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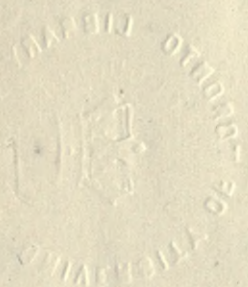
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## CONTENTS OF VOLUME XIX

### JANUARY, 1917. NUMBER 1

	PAGE
THE CLINICAL VALUE OF AMBARD'S COEFFICIENT OF UREA EXCRETION. D. SCLATER LEWIS, M.D., BALTIMORE.....	1
THE ACTION OF THE SEVERAL "FEMALE REMEDIES" ON STRIPS OF THE EXCISED HUMAN UTERUS. J. D. PILCHER, M.D., OMAHA.....	53
THE PRESENT SIGNIFICANCE OF THE AMINO-ACIDS IN PHYSIOLOGY AND PATHOLOGY. DONALD D. VAN SLYKE, PH.D., NEW YORK.....	56
PROGRESSIVE MUSCULAR DYSTROPHY AS AN ENDOCRINE DISEASE. WALTER TIMME, M.D., NEW YORK.....	79
BLOOD CHANGES IN ALBINO RATS FOLLOWING REMOVAL OF THE SPLEEN. C. C. WOLFERTH, M.D., PHILADELPHIA.....	105
EXPERIMENTS ON THE ORIGIN AND CONDUCTION OF THE CARDIAC IMPULSE. VII. SINOVENTRICULAR AND SINO-AURICULAR HEART-BLOCK. J. A. E. EYSTER, M.D., AND WALTER J. MEEK, PH.D., MADISON, WIS.....	117
THE OCCURRENCE OF NUCLEAR CHANGES IN THE RED BLOOD CELLS FOLLOW- ING SPLENECTOMY. QUINTER O. GILBERT, M.D., ANN ARBOR, MICH...	140
THE EFFECT OF HEAT AND CONTINUOUS INCANDESCENT ELECTRIC LIGHT IN EXPERIMENTAL ARTHRITIS. WALTER E. SIMMONDS, M.D., AND J. J. MOORE, M.S., M.D., CHICAGO.....	153
FURTHER QUANTITATIVE STUDY OF THE DUODENAL BLOOD-DERIVED PIGMENTS. J. P. SCHNEIDER, M.D., MINNEAPOLIS.....	156

### FEBRUARY, 1917. NUMBER 2

	PAGE
OSTEOGENESIS IMPERFECTA. JULIUS H. HESS, M.D., CHICAGO.....	163
HEREDITARY HEMORRHAGIC TELANGIECTASIA, WITH REPORT OF THREE FAM- ILIES AND A REVIEW OF THOSE PREVIOUSLY RECORDED. WALTER R. STEINER, M.D., HARTFORD, CONN.....	194
A CLINICAL STUDY OF THE SECRETIONS ON THE PROXIMAL AND DISTAL SIDES OF THE PYLORUS. A. S. ROBINSON, M.D., CLEVELAND.....	220
THE RELATION OF HYPERTROPHIC OSTEO-ARTHROPATHY TO PULMONARY TUBERCULOSIS. LEO KESSEL, M.D., NEW YORK.....	239
TYPHOIDIN QUOTIENTS. AN ANALYSIS OF THE FACTORS OF UNCERTAINTY IN THE CUTANEOUS TYPHOIDIN TEST. EUGENE S. KILGORE, SAN FRANCISCO .....	263
A COMPARISON OF TWO METHODS OF VACCINATING AGAINST TYPHOID FEVER. EUGENE S. KILGORE, SAN FRANCISCO.....	276
THE AGGLUTININS AND COMPLEMENT-FIXING ANTIBODIES IN THE SERUM OF PERSONS VACCINATED AGAINST TYPHOID FEVER. K. F. MEYER AND EUGENE S. KILGORE, SAN FRANCISCO.....	293
d-GLUCOSE TOLERANCE IN HEALTH AND DISEASE. RUSSELL M. WILDER, M.D., AND W. D. SANSUM, M.D., CHICAGO.....	311



# CONTENTS OF VOLUME XIX

## MARCH, 1917. NUMBER 3

PAGE

THE EFFECT OF BANDAGING OF THE LEGS ON THE RATE OF BLOOD FLOW IN THE FEET. G. N. STEWART, M.D., CLEVELAND.....	335
THE BILE CONTENT OF THE BLOOD IN PERNICIOUS ANEMIA. M. A. BLANKENHORN, M.D., CLEVELAND.....	344
REFRACTOMETRIC STUDIES OF SERUM PROTEINS IN NEPHRITIS, CARDIAC DECOMPENSATION, DIABETES, ANEMIA, AND OTHER CHRONIC DISEASES. ALBERT H. ROWE, M.D., OAKLAND, CALIF.....	354
BACTERIOLOGIC STUDIES IN SUBACUTE STREPTOCOCCUS ENDOCARDITIS. RALPH A. KINSELLA, M.D., NEW YORK.....	367
BACTERIOLOGIC STUDIES IN ACUTE RHEUMATIC FEVER. HOMER F. SWIFT, M.D., AND RALPH A. KINSELLA, M.D., NEW YORK.....	381
THE MALONE-KIUTSI REACTIONS IN PREGNANCY AND CANCER. SAMUEL BERKOWITZ, M.D., NEW YORK.....	397
THE RELATION OF PREGNANCY AND CHILDBIRTH TO PELLAGRA IN WOMEN. J. F. SILER, M.D., P. E. GARRISON, M.D., AND W. J. MACNEAL, M.D., NEW YORK .....	404
FURTHER STUDIES ON TYPHOIDIN. JOHN N. FORCE, M.D., AND IDA M. STEVENS, M.A., BERKELEY, CALIF.....	440
ON THE RELIABILITY OF THE WASSERMANN REACTION. A STUDY OF THE SOURCES OF ERROR AND AN ATTEMPT TO STANDARDIZE THE TECHNIC. REUBEN OTTENBERG, M.D., NEW YORK.....	457
THE BACTERIOLOGY OF THE URINE IN FOCAL INFECTIONS; ITS RELATION TO NEPHRITIS. GEORGE F. DICK, M.D., AND GLADYS R. DICK, M.D., CHICAGO	493

## APRIL, 1917. NUMBER 4

PAGE

THE EFFECT OF MUSCULAR WORK, DIET AND HEMOLYSIS ON THE SERUM PROTEINS. A. H. ROWE, M.D., OAKLAND, CALIF.....	499
THE CAUSES OF THE VARIATION IN THE CONCENTRATION OF UREA IN THE BLOOD OF YOUNG, HEALTHY ADULTS. T. ADDIS, M.D., AND C. K. WATANABE, M.D., SAN FRANCISCO.....	507
AUTOTRANSPLANTATION AND HOMOTRANSPLANTATION OF THE THYROID GLAND, USING THE CAPSULE AS THE SEAT OF TRANSPLANTATION. J. H. HESS, M.D., AND A. A. STRAUSS, M.D., CHICAGO.....	518
THE EFFECT OF SALICYLATES ON EXPERIMENTAL ARTHRITIS IN RABBITS. B. FANTUS, M.D., W. E. SIMMONDS, M.D., AND J. J. MOORE, M.D., CHICAGO	529
ROENTGENOGRAPHY OF THE LUNGS. ROENTGENOGRAPHIC STUDIES IN LIVING ANIMALS AFTER INTRATRACHEAL INJECTIONS OF IODOFORM EMULSION. C. A. WATERS, M.D., S. BAYNE-JONES, M.D., BALTIMORE, AND L. G. ROWNTREE, M.D., MINNEAPOLIS.....	538
A CASE OF INFUNDIBULAR TUMOR IN A CHILD, CAUSING DIABETES INSIPIDUS WITH TOLERANCE OF ALCOHOL. L. NEWMARK, M.D., SAN FRANCISCO..	550
THE REACTIVATED THYMUS. G. H. HOXIE, M.D., KANSAS CITY, MO.....	564
A STUDY OF THE DIASTATIC ACTIVITY OF THE URINE AND FECES WITH SPECIAL REFERENCE TO DISEASES OF THE PANCREAS. C. W. MCCLURE, M.D., AND J. H. PRATT, M.D., BOSTON.....	568
THE SUPRARENAL SYSTEM AND CARBOHYDRATE METABOLISM. G. M. MACKENZIE, M.D., NEW YORK.....	593
A STUDY OF ETHYLHYDROCUPREIN (OPTOCHIN) IN THE TREATMENT OF ACUTE LOBAR PNEUMONIA. H. F. MOORE, M.B., B.CH., AND A. M. CHESNEY, M.D., NEW YORK.....	611



# CONTENTS OF VOLUME XIX

## MAY, 1917. NUMBER 5. PART I

	PAGE
AN EXPERIMENTAL TEST OF THE RELATION OF SEWAGE DISPOSAL TO THE SPREAD OF PELLAGRA. J. F. SILER, M.D., P. E. GARRISON, M.D., AND W. J. MACNEAL, M.D., NEW YORK.....	683
INTESTINAL EOSINOPHILIA, WITH REPORT OF A CASE. G. D. BARNETT, M.D., SAN FRANCISCO.....	695
THE SIGNIFICANCE OF EMBRYONAL FAT CELLS IN CERTAIN PATHOLOGIC CONDITIONS. D. SYMMERS, M.D., AND A. FRASER, M.D., NEW YORK.....	699
EXPERIMENTAL APPENDICITIS. J. W. McMEANS, M.D., NEW YORK.....	709
TRANSIENT HEART BLOCK — ELECTROCARDIOGRAPHIC STUDIES. E. B. KRUMBHAAR, M.D., PHILADELPHIA.....	750
THE CARBON DIOXID CONTENT OF BLOOD AND OF ALVEOLAR AIR IN OBSTRUCTED EXPIRATION. E. D. FRIEDMAN AND H. C. JACKSON, NEW YORK.....	767
A STUDY OF BLOOD SUGAR. A COMPARISON OF THE TOLERANCE FOR GLUCOSE IN DIABETIC AND NORMAL SUBJECTS. R. CUMMINGS, M.D., AND G. PINESS, M.D., LOS ANGELES.....	777
THE HUMAN AND ANIMAL LIVER AFTER ALCOHOL. F. A. McJUNKIN, M.D., MILWAUKEE, WIS.....	786
THE INFLUENCE OF THE RADIATIONS FROM KROMAYER'S MERCURY QUARTZ LAMP ON THE CEREBRAL CORTEX (ANIMAL EXPERIMENTS). H. WAGO, M.D., TOKIO, JAPAN.....	801
THE REFLEX ACTION OF VOLATILE IRRITANTS ON THE CIRCULATION. C. C. LIEB, M.D., AND W. W. HERRICK, M.D., NEW YORK.....	811

## MAY, 1917. NUMBER 5. PART II

	PAGE
CLINICAL CALORIMETRY. NINETEENTH PAPER. THE BASAL METABOLISM OF OLD MEN. J. C. AUB, M.D., AND E. F. DuBOIS, M.D., NEW YORK.....	823
CLINICAL CALORIMETRY. TWENTIETH PAPER. THE EFFECT OF CAFFEIN ON THE HEAT PRODUCTION. J. H. MEANS, M.D., BOSTON, J. C. AUB, M.D., AND E. F. DuBOIS, M.D., WITH THE TECHNICAL ASSISTANCE OF G. F. SODERSTROM, NEW YORK.....	832
CLINICAL CALORIMETRY. TWENTY-FIRST PAPER. THE BASAL METABOLISM OF DWARFS AND LEGLESS MEN, WITH OBSERVATIONS ON THE SPECIFIC DYNAMIC ACTION OF PROTEINS. J. C. AUB, M.D., AND E. F. DuBOIS, M.D., WITH THE TECHNICAL ASSISTANCE OF G. F. SODERSTROM, NEW YORK.....	840
CLINICAL CALORIMETRY. TWENTY-SECOND PAPER. THE RESPIRATORY METABOLISM IN NEPHRITIS. J. C. AUB, M.D., E. F. DuBOIS, M.D., WITH THE TECHNICAL ASSISTANCE OF G. F. SODERSTROM, NEW YORK.....	865
CLINICAL CALORIMETRY. TWENTY-THIRD PAPER. THE EFFECT OF ROENTGEN-RAY AND RADIUM THERAPY ON THE METABOLISM OF A PATIENT WITH LYMPHATIC LEUKEMIA. J. B. MURPHY, M.D., NEW YORK; J. H. MEANS, M.D., BOSTON, AND J. C. AUB, M.D., NEW YORK.....	890
CLINICAL CALORIMETRY. TWENTY-FOURTH PAPER. METABOLISM IN THREE UNUSUAL CASES OF DIABETES. F. C. GEPHART, PH.D., J. C. AUB, M.D., E. F. DuBOIS, M.D., GRAHAM LUSK, Sc.D., WITH THE TECHNICAL ASSISTANCE OF G. F. SODERSTROM, NEW YORK.....	908
CLINICAL CALORIMETRY. TWENTY-FIFTH PAPER. THE WATER ELIMINATION THROUGH SKIN AND RESPIRATORY PASSAGES IN HEALTH AND DISEASE. G. F. SODERSTROM AND E. F. DuBOIS, M.D., NEW YORK.....	931



# CONTENTS OF VOLUME XIX

JUNE, 1917. NUMBER 6

	PAGE
HAY-FEVER AND HAY-FEVER POLLENS. WILLIAM SCHEPPEGRELL, M.D., NEW ORLEANS .....	959
THE SYSTOLIC BLOOD PRESSURE FOLLOWING EXERCISE; WITH REMARKS ON CARDIAC CAPACITY. D. L. RAPPORT, BOSTON.....	981
A NEW INTERPRETATION OF THE PATHOLOGIC HISTOLOGY OF HODGKIN'S DIS- EASE. DOUGLAS SYMMERS, M.D., NEW YORK.....	990
THE TREATMENT OF SYPHILIS OF THE CENTRAL NERVOUS SYSTEM. A COMPARISON OF MERCURIALIZED SERUM AND SALVARSANIZED SERUM. DAVID A. HALLER, M.D., BOSTON.....	997
THE SALICYLATES. VI. RENAL FUNCTION AND MORPHOLOGIC CHANGES IN ANIMALS FOLLOWING THE ADMINISTRATION OF SALICYLATE. PAUL J. HANZLIK, M.D., AND HOWARD T. KARSNER, M.D., CLEVELAND.....	1016
THE SALICYLATES. VII. FURTHER OBSERVATIONS ON ALBUMINURIA AND RENAL FUNCTIONAL CHANGES FOLLOWING THE ADMINISTRATION OF FULL THERAPEUTIC DOSES OF SALICYLATE. PAUL J. HANZLIK, M.D., R. W. SCOTT, M.D., AND T. W. THOBURN, A.B., CLEVELAND.....	1029
THE INFLUENCE OF NONSPECIFIC SUBSTANCES ON INFECTIONS. JAMES W. JOBLING, M.D., NASHVILLE, TENN.....	1042
THE EFFECT OF PITUITARY INJECTIONS ON THE BLOOD PRESSURE OF FEBRILE PATIENTS. HARRY B. SCHMIDT, ANN ARBOR, MICH.....	1059
DIMINISHED BLOOD PLATELETS AND MARROW INSUFFICIENCY. A CLASSI- FICATION AND DIFFERENTIAL DIAGNOSIS OF PURPURA HEMORRHAGICA, APLASTIC ANEMIA AND ALLIED CONDITIONS. GEORGE R. MINOT, M.D., BOSTON .....	1062
THE REACTION OF THE CEREBROSPINAL FLUID. PRELIMINARY REPORT ON HYDROGEN-ION CONCENTRATION AS DETERMINED BY THE COLORIMETRIC METHOD. LLOYD D. FELTON, M.D., R. G. HUSSEY, M.D., AND S. BAYNE-JONES, M.D., BALTIMORE.....	1085



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## THE CLINICAL VALUE OF AMBARD'S COEFFICIENT OF UREA EXCRETION \*

D. SCLATER LEWIS, M.D.

BALTIMORE

In the study of any metabolic process account has to be taken of the excretion of waste products, the food intake, and also the various changes which this food undergoes in the body before the end-products are ready for excretion. The last constitutes the intermediary metabolism, of which our knowledge is still very imperfect. If any one of these groups of processes is studied to the exclusion of the others, very erroneous ideas regarding the metabolism will be obtained. Likewise, in the study of renal function chemical observations of any one of these processes (nitrogen intake or output, or retention of waste nitrogen) will fail to give accurate information unless something is known of the other metabolic factors. Little value can be attached to the finer changes in the level of the blood urea, or total nonprotein nitrogen, as a gage of renal function, unless the protein intake is known. Widal<sup>1</sup> suggested this precaution several years ago, but reference to the patient's diet is of rare occurrence in studies of renal function by the tests of retention. The necessity of such information is constantly increasing because of the popularity of low protein diets in the treatment of the azotemic type of nephritis. With the diet devised by Mosenthal and Richards<sup>2</sup> reductions of 75 per cent. in the level of the total nonprotein nitrogen are of frequent occurrence, so that values of from 30 to 35 mg. are not unusual, even in severe nephritis. An estimation of the protein intake should be given, therefore, in every case in which conclusions regarding renal function are being drawn from studies of the waste nitrogen of the blood.

The excretion of various substances in the urine has also been used in testing renal function. These tests are of a qualitative and quantitative nature.

\* Submitted for publication July 21, 1916.

\* From the Medical Clinic of the Johns Hopkins Hospital, Baltimore.

1. Widal and Javal: *Compt. rend. Soc. de biol.*, 1904, **56**, 301.

2. Mosenthal, H. O., and Richards, A. E.: *THE ARCHIVES INT. MED.*, 1916,

To make a qualitative test of renal function, a somewhat copious diet<sup>3</sup> is given at fixed intervals, and the variation in the volume and specific gravity of the urine is studied in two-hour periods during the day. The night voiding is collected in one specimen and studied as a whole. The normal reaction to these three meals is a markedly variable specific gravity during the day, with an output of from 65 to 75 per cent. of the water during this period and the excretion of a small amount of highly concentrated urine at night. No attention is paid to the relative intake and output of nitrogen and salt, their concentration being the criterion on which a decision is based. These test diets give valuable information in the study of renal function in nephritis, both in the early and late stages of the disease.

Ambard<sup>4</sup> has followed the same general principles in his studies of renal function by the determination of the kidney's maximal concentrating power. He has found a diminution of the maximal concentration to be one of the earliest signs of impaired function. With each advance in the renal injury there was a corresponding diminution in the maximal concentration.

Siebeck<sup>5</sup> and his associates have also applied the same principles in their studies of the concentrating and diluting powers of the kidney. Their results confirm Ambard's findings.

There is one possible exception to the conclusions stated above. In severe anemia, with a marked hydremia of the blood, a low, fixed specific gravity is a constant finding. Whether hyposthenuria in such cases is of renal origin or a result of the constitutional changes has not been decided. A few cases show a decreased phenolsulphonephthalein output and an increased coefficient of urea excretion, pointing to definite renal involvement. In other cases these changes are absent and no direct evidence of kidney insufficiency can be obtained.

As quantitative tests to determine the reaction of the kidney to an overload of the ordinary metabolic products, large amounts of urea, sodium chlorid and water are added to a standard diet and the results are studied. No attention is paid to the concentrating power of the kidney, and all normal requirements are satisfied by the excretion of a certain proportion of the added substances within a given time.

The phenolsulphonephthalein test is the only test dependent on the excretion of foreign substances at present in common use. It requires no description.

According to Mosenthal and Richards<sup>2</sup> very little information

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3. Mosenthal, H. O.: *THE ARCHIVES INT. MED.*, 1915, **16**, 733.

4. Ambard, L.: *Physiologie normale et pathologique des reins*, Paris, 1914, p. 9.

5. Hefter and Siebeck: *Deutsch. Arch. f. klin. Med.*, 1914, **114**, 497. Doll and Siebeck: *Ibid.*, **116**, 549.



regarding the intermediary metabolism can be obtained from a determination of the nitrogen balance. Even in severe nephritis there is no relation between the amounts of nitrogen retained and the level of the waste nitrogen in the blood. The discrepancy between the intake and output cannot be regarded as an evidence of renal insufficiency. The nitrogen balance cannot be used for the quantitative study of renal function.

An ideal quantitative method, however, would be a direct comparison of the processes of the intermediary metabolism with those of excretion, in other words, a comparison of the concentration of the waste nitrogen in the blood with its rate of excretion in the urine. Under such conditions the unknown factors would be reduced to the rate of blood flow through the kidney and the functional activity of that organ. Such studies have been made possible by the work of Ambard and his associates.

Lamy and Mayer<sup>6</sup> endeavored to compare the concentration of urea in the blood with the rate of excretion in the urine. They did not recognize the importance of the rate of blood flow, and consequently were not able to find any relation between the two values. Five years later Ambard and Moreno<sup>7</sup> announced their laws of renal function. They were three in number, and reduced the study of kidney activity to a physicochemical basis. The blood urea was regarded as a stimulus acting on the renal cells. The rate of excretion of urea was the response of the kidney to that stimulus. In their opinion the rate of circulation through the kidney was the chief factor governing the concentration of the urine. A dilute urine was a sign of a high rate of blood flow, while a diminished blood supply was shown by the increase in the concentration of the urine and a consequent diminished output of water and of urea.

The first law dealt with the relation of the rate of output of urea to the concentration of urea in the blood. The rate of output was found to vary directly with the square of the concentration of urea in the blood, if the concentration of urea in the urine remained constant. In other words, if the quantity of urea in the blood were doubled, the amount excreted in a given time would be quadrupled.

According to the second law, the rate of excretion of urea varied inversely with the square root of the concentration of urea in the urine, if the blood urea remained constant. Under these conditions a quadrupling of the concentration would result in a halving of the rate of output.

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6. Lamy and Mayer: *Jour. de physiol. et de pathol. gén.*, 1905, **7**, 679.

7. Ambard, L.: *Compt. rend. Soc. de biol.*, 1910, **69**, 411, 506. Ambard, L., and Weill, A.: *Jour. de physiol. et de pathol. gén.*, 1912, **32**, 217.

The third law was a combination of the first and second. If the concentration of the urea in the blood and urine varied simultaneously, then the rate of output would vary directly as the square of the concentration of urea in the blood, and inversely as the square root of that in the urine.

The following formula, used in calculating the coefficient, is derived from the third law by the addition of correction factors for the patient's weight and for a standard urinary concentration of 25 gm. of urea per liter.

$$K = \sqrt{\frac{U_r}{D \times \frac{70}{P} \times \frac{\sqrt{C}}{\sqrt{25}}}}$$

$K$  = coefficient of urea excretion.

$U_r$  = grams of urea per liter of blood.

$D$  = output of urea in grams per twenty-four hours.

$P$  = weight of the patient in kilograms.

$C$  = grams of urea per liter of urine.

70 = standard weight.

25 = standard concentration of urea in the urine.

All weights and concentrations are compared to the standards of 70 and 25.

The normal value of the constant is from 0.06 to 0.09. With a decreasing kidney efficiency there is a rise in the constant, and with an increasing function the coefficient falls.

The analytical methods used by the French had been so inaccurate that this work remained practically unknown in this country until McLean and Selling<sup>8</sup> repeated the work, using more accurate methods, and found the coefficient to have the same degree of constancy that Ambard had found four years previously.

In a second paper McLean<sup>9</sup> discusses the laws of function somewhat fully. He concludes that "the excretion of urea and chlorids in the normal individual is carried out according to definite laws capable of numerical expression." He finds, however, that in normal, healthy adults the laws of function are not followed so closely as by individuals living under conditions of ward routine. In the former he finds a tendency to a low concentration of blood urea, and a greater output of urea than is justified by the laws of function. In the latter cases, the blood urea is uniformly higher and the laws of function are followed more closely. These variations from the laws of function are considered merely as signs of the ready response met with in healthy young adults, living an active life. The validity of this conclusion is strengthened by the fact that variations from the laws of function are always in the direction of a hyperfunction of the kidney.

8. McLean, F. C., and Selling, L.: *Jour. Biol. Chem.*, 1914, **19**, 31.

9. McLean, F. C.: *Jour. Exper. Med.*, 1915, **22**, 212.



Recently, Addis and Watanabe<sup>10</sup> have applied Ambard's laws to the results obtained in the studies of urea excretion in normal students. They find a very marked discrepancy between the laws of function and the results actually obtained. The most serious differences are seen in the second law, in which variations of 150 per cent. from the theoretical values are not uncommon. In the application of the first law the variations are of a less serious character. They conclude that the variations in urea output cannot be explained by the concentration of urea in blood and urine. The subjects of these experiments are of the same type as those which McLean finds to show the greatest variations from normal.

In the present series of forty normal hospital patients, it was possible to test the validity of Ambard's laws of function in a number of instances.

A number of cases had identical concentrations of urea in the blood. In these cases, according to Ambard's second law, the expression

$$\text{Rate of output} \times \sqrt{\text{Conc.}^n \text{ of urea in the urine}} \times \frac{70}{\text{Kg. of body wt.}}$$

should be a constant in each group of cases. The maximum error in this law was 47 per cent. but the average error was 20 per cent. This was decidedly lower than those reported by Addis and Watanabe.

In the same way, twelve groups of from two to five cases each were found having the same concentrations of urea in the urine. According to the first law

$$\sqrt{\frac{\text{Concentration of urea in blood}}{\text{Rate of output} \times \frac{70}{\text{Kg. of body wt.}}}}$$

should be a constant for each group. The adherence to this law of function was remarkably good. In very few of the cases did the factor show a variation of more than 10 per cent. from a normal value. The maximum variation was 30 per cent., the average in the neighborhood of 8 per cent.

It is evident from the above that the laws of function are followed more closely by individuals living under routine conditions than by those living on a more luxurious diet and subject to the strain of active life.

It has been claimed<sup>10</sup> that the mathematical form of the coefficient is the real basis of its constancy and that the laws of function play absolutely no rôle in the attainment of this end. Addis and Watanabe claim that *Ur* is a relatively constant value and that it is the dominant factor in keeping the coefficient constant. On the other hand, *D* and *C* are the factors which are subject to considerable variation. The effects

10. Addis, T., and Watanabe, C. K.: Jour. Biol. Chem., 1916, **24**, 203.

of these variations are minimized by the fact that the greater variant is present as its fourth root, and the lesser as its square root. While their assumption is partly true, the fact remains that the blood urea is not always a constant value. In a series of nephritics here presented there are instances of enormous variations in the blood urea, without any corresponding change in the coefficient of urea excretion. While, strictly speaking, the laws of function may be mathematically inaccurate, and the formula from which the coefficient is calculated may tend to hide these inaccuracies, still there is a very definite relationship between the concentration of the urea in the blood and in the urine and its rate of excretion. Under similar conditions of urine concentration the rate of urea excretion is always greater with a high concentration of blood urea than with a low one. Similarly, in the presence of a stationary blood urea concentration the amounts of urea excreted under conditions of polyuria and low urinary concentration are greater than when the volume is small and the concentration high.

In spite of the lack of mathematical accuracy, the normal coefficient of urea excretion falls within comparatively narrow limits.

Objection has been made to the original form of the coefficient because the normal value is not a whole number, and because a diminishing renal function causes a rise in the coefficient. Balavoine and Onfray<sup>11</sup> have advocated the use of a formula giving the normal coefficient a value of 1, and so arranging the equation that a diminution in renal function would cause a drop in the constant. Their suggestion was not well received in France.

Ambard<sup>12</sup> has suggested a means of calculating the functional capacity of a damaged kidney in terms of percentage of the normal. For example, if, after the injection of urea, a normal individual presents a blood urea of 0.7 gm. per liter, his rate of output should be 100 gm. per twenty-four hours, at the standard concentration of 25 per 1,000. This gives a coefficient of 0.07. If a nephritic with a similar concentration of blood urea excretes only 25 gm. at the standard concentration, his rate of output is reduced to a quarter of the normal. His coefficient under these conditions would be 0.14, because

$$K = \frac{\text{Blood urea}}{\sqrt{\text{Rate of output}}} = \frac{0.7}{\sqrt{25}} = 0.14$$

Again, if the rate of output were reduced to one-ninth the normal, or 11.11 gm., the coefficient would be

$$\frac{0.7}{\sqrt{11.11}} = 0.21.$$

11. Balavoine and Onfray: *Presse méd.*, 1912, **21**, 786.

12. Ambard: *Physiologie normale et pathologique des reins*, Paris, 1914, p. 200.



The absolute functional value of the kidney can also be obtained from the coefficient by the use of the following equation:

$$\left[ \frac{\text{Normal coefficient}}{\text{Pathologic coefficient}} \right]^2 = \text{Functional value of the kidney in terms of normal.}$$

If we apply this formula to the first case,  $\left[ \frac{0.07}{0.14} \right]^2 = 0.25$ , that is, the functional capacity of the kidney is reduced to one fourth of the normal value. This corresponds to the theoretical diminution in the rate of output. The same applies to the second case.  $\left[ \frac{0.07}{0.21} \right]^2 = 0.11$ , or one ninth of the normal value.

McLean's index<sup>9</sup> is based on this modification of the original coefficient. In his opinion the average normal coefficient is 0.08, and this is used in the fraction

$$\left[ \frac{\text{Normal } K}{\text{Pathologic } K} \right]^2$$

instead of 0.07. To simplify calculation, the expression

$$\frac{Ur}{\sqrt{D \times \frac{70}{P} \times \frac{\sqrt{C}}{\sqrt{25}}}}$$

is substituted for the pathologic constant, and to avoid the use of decimals, the resultant is multiplied by 100. The formula

$$I = \frac{D \sqrt{C} \times 8.96}{Wt. \times Ur^2}$$

is obtained by simplifying the above fraction. *I* is the index of urea excretion. The normal value of the index is 100 and the maximal range is between 80 and 250. The index expresses the functional capacity of the kidney in percentage of normal.

So much work has been published in the European journals in which the original coefficient is quoted, and the normal index shows such enormous variations that it seems unnecessary to have added this new value. However, the index of urea excretion has been included in the tables for purposes of comparison.

In the present series the total nonprotein nitrogen and the urea nitrogen of the blood, and the excretion of phenolsulphonaphthalein will be compared with the coefficient of urea excretion.

#### METHODS

The concentration of urea in both urine and blood normally shows great variations in the course of the day, particularly in relation to meals. This makes it impossible to compare the concentration of

blood urea at any one time with the daily output of urea. Ambard avoided this difficulty by studying the renal activity over a comparatively short period, the time chosen being as remote as possible from a meal rich in protein. The majority of our studies were made two to three hours after breakfast. The amount of urea excreted in the test interval, calculated for a twenty-four-hour period, therefore, represents the quantity of urea which would be eliminated in a day, provided the concentration of the urea in the blood and urine remained constant during the whole twenty-four hours. The latter is a condition impossible of attainment under any circumstances.

Theoretically, the shorter the period of observation, the more accurate should be the results. In the wards of a general hospital, however, it is impossible to depend on the absolute punctuality with which specimens are obtained. To lessen the effect of this error, a longer period has been used than that suggested by Ambard and McLean.

Half an hour before the first voiding, the patient is given 200 c.c. of water. This insures a free diuresis during the test period, and facilitates the prompt collection of specimens. The first specimen of urine is discarded. Exactly two hours after the first voiding, the patient again empties the bladder. The second specimen represents the amount of urine secreted during the period of observation. It is measured carefully to within 1 c.c. and is used for analysis. Midway between the voidings 10 c.c. of blood are drawn into a tube which contains a drop of saturated potassium oxalate solution to prevent clotting. The concentration of urea in this specimen represents the average level of the blood urea during the test period.

The urea in the blood and urine may be determined accurately by Marshall's urease methods.<sup>13</sup> The procedures are simple and rapid. Duplicate determinations are unnecessary after one has become familiar with the technic.<sup>14</sup>

From the results of the foregoing analyses the twenty-four-hour output of urea ( $D$ ) is calculated. The concentration of the urea in the blood ( $Ur$ ) and in the urine ( $C$ ) and also the patient's weight in kilograms ( $P$ ) are substituted in the formula

$$K = \frac{Ur}{\sqrt{D \times \frac{70}{P} \times \frac{\sqrt{C}}{\sqrt{25}}}}$$

13. Marshall, E. K.: Jour. Biol. Chem., 1913, **14**, 283; *ibid.*, **15**, 487.

14. Urease-Dunning tablets have been used exclusively. Two 25-mg. tablets were found sufficient to digest 5 c.c. of urine in three hours at 37 C., while the urea in 5 c.c. of blood was completely decomposed by three tablets in thirty minutes at 37 C. Each batch of enzyme should be tested for preformed ammonia and a suitable correction applied, if necessary.



The solution of the equation gives the value of  $K$ , which is the coefficient of urea excretion.<sup>15</sup>

In determining the total nonprotein nitrogen of the blood Folin's<sup>16</sup> method of precipitation and digestion was used. A combination of heat and aeration was employed in removing the ammonia from the digestion mixture.

In making the phenolsulphonephthalein test Rowntree and Geraghty's<sup>17</sup> technic was followed in the determination of the rate of excretion. The two-hour output was recorded, as no marked significance could be attached to the hourly excretions. In cases with marked edema the dye was injected intravenously and the amount excreted in the first hour was recorded.

#### TYPES OF CASES STUDIED

The series contains a considerable number of individuals with no cardiorenal changes, mostly hospital patients. Cases of fever, hyperthyroidism and myxedema are included. Cardiac cases are presented in the stage of decompensation, and also when well compensated. The group of nephritics contains examples of all the usual types of nephritis, both acute and chronic. There are cases of mercury bichlorid and salvarsan poisoning, and also three cases of polycystic kidney.

#### NORMAL CASES

With few exceptions, the cases presented in this group are ward patients in whom the cardiorenal system is normal on routine examination. All persons convalescing from acute infections, diabetics with severe acidosis, and cases of hyperthyroidism are excluded, even though the urine be free of albumin and casts.<sup>18</sup>

Ambard gives 0.07 as the normal value of the coefficient, with normal range of from 0.06 to 0.08. In a series of 107 determinations, McLean finds the maximum normal range of the coefficient to be from 0.05 to 0.09 (index 235-80). He considers any coefficient above 0.09 (index below 80) distinctly abnormal, unless the reduced rate of urea excretion can be accounted for. An insufficient water intake is a possible cause of increased values of the coefficient in quite normal

15. The use of an ordinary 10-inch slide rule greatly facilitates the calculation, reducing the whole operation to approximately sixty seconds. McLean (Footnote 9) has devised a special slide rule for the determination of his index, but this is unnecessary in the calculation of the original coefficient.

16. Folin, O., and Denis, W.: *Jour. Biol. Chem.*, 1912, **11**, 527.

17. Rowntree, L. G., and Geraghty, T.: *THE ARCHIVES INT. MED.*, 1912, **10**, 284.

18. It has been found that many of these cases do not have a normal renal function. The phenolsulphonephthalein excretion is increased, and the rate of urea excretion is much greater than is justified by the concentration of urea in the blood.

TABLE 1.—NORMAL CASES

Case Number	Medical Number	Urea N Mg. per 100 C.c. Blood	Coefficient	Index	'Phthal- ein Output, % 2 hr.	Diagnosis
301	.....	9	0.053	228	55	Psychoneurosis
302	H. G.	12	0.054	220	..	
303	.....	14	0.056	204	..	Primary anemia
304	34128	20	0.058	190	..	
305	34865	10	0.062	167	62	Psychoneurosis
306	D. S. L.	13	0.062	167	60	
307	D. S. L.	10	0.066	147	..	
308	D. S. L.	7	0.058	190	..	
309	34717	16	0.063	161	..	Primary anemia
310	34331	14	0.064	156	57	Cerebral arteriosclerosis
312	35118	10	0.067	143	56	Neurasthenia
314	34680	14	0.072	124	66	
315	33714	14	0.072	124	76	Syphilis, tertiary
316	33629	17	0.073	120	..	Primary anemia
317	33692	16	0.074	117	60	Gastroptosis
319	34967	19	0.075	114	65	Diabetes mellitus
320	35011	11	0.076	111	47	Colloid goiter
321	33996	16	0.077	108	67	Multiple sclerosis
322	34638	14	0.077	108	..	Syphilis
323	34817	11	0.077	108	..	Primary anemia
325	34716	20	0.078	105	..	
326	34634	13	0.079	102	50	Neurasthenia
327	35097	18	0.079	102	60	Syphilis, tertiary
329	34674	23	0.081	97	66	
330	35102	14	0.082	95	51	Obesity
332	34915	11	0.082	95	57	Pyloric stenosis
333	35315	13	0.083	93	55	Neurasthenia
335	.....	16	0.085	89	60	Chronic appendicitis
336	.....	10	0.087	85	67	
337	34195	17	0.087	85	55	General paresis
339	34709	17	0.092	76	55	Intestinal parasitism
340	34657	20	0.092	76	79	Psychoneurosis



persons. These effects of dehydration are guarded against by the ingestion of water before the test period. The findings in our series of forty normal persons are given in Table 1. They are arranged in the order of increasing coefficients. The concentration of blood urea is given in every case, and the phenolsulphonephthalein output is included whenever the figures are available.

The coefficients vary from the minimum of 0.053 to a maximum of 0.092, with an average value of 0.074. This is somewhat lower than the average normal (0.08) given by McLean.

Of the forty cases, twenty-two, or 56 per cent., had coefficients between 0.065 and 0.085, while 82.5 per cent. had values between 0.06 and 0.09.

Normal values of blood urea have been found to vary between narrow limits. Folín and Denis<sup>19</sup> give the normal variation as from 11 to 14 mg. of urea nitrogen per 100 c.c. However, when clinical material without renal involvement is studied, the results are found to be much higher. According to Folín and Denis,<sup>19</sup> the usual range in such persons is from 10 to 26 mg. per 100 c.c.

In the present series the blood urea nitrogen varies from 7 to 23 mg. per 100 c.c., with an average of 14.4 mg. Of the forty cases, less than 50 per cent. have a concentration of urea nitrogen between 10 and 15 mg., and fully 40 per cent. have a concentration above 15 mg. While relatively few normals present concentrations above the upper normal limit of 25 mg. per 100 c.c. suggested by Rowntree and Marshall,<sup>20</sup> still the urea nitrogen approaches this limit in a great number of persons. McLean has found that the laws of function are followed much more closely in cases with a blood urea nitrogen above 14 mg. than in those having a lower concentration of urea in the blood.

Reference to Table 1 will show the entire absence of relationship between the level of the coefficient and that of the blood urea. In many cases the higher values of blood urea are associated with very low coefficients, and vice versa. (In Case 4,  $U-N = 20$  mg.,  $K = 0.058$ ; in Case 24,  $U-N = 8$  mg.,  $K = 0.078$ ; in Case 25,  $U-N = 20$  mg.,  $K = 0.078$ .) These findings offer definite proof that the level of  $Ur$  is not the dominant factor in the formula

$$\sqrt{\frac{Ur}{D \times \frac{70}{P} \times \frac{\sqrt{C}}{25}}}$$

It is evident that the effects of the variations in  $Ur$  are adequately met by corresponding variations in the rate of output. The narrow limits

19. Folín, O., and Denis, W.: Jour. Biol. Chem., 1913, **14**, 29.

20. Rowntree, L. G., and Marshall, E. K.: Tr. Assn. Am. Phys., 1914.

of the normal coefficient (from 0.06 to 0.09) show that while the laws may not be mathematically exact, still they are at least correct in principle.

According to the originators of the phenolsulphonephthalein test, normal persons should excrete from 50 per cent. to 75 per cent. of the dye in two hours. The phenolsulphonephthalein excretion is given in twenty-eight of the forty normals. Two persons failed to excrete 50 per cent. or over, but in both instances the output (47 per cent.) was extremely close to that figure. The output ranged from 47 per cent. to 79 per cent. with an average of 60.2 per cent. There was no absolute relationship between the rate of output and the level of the coefficient, the high and low excretions of the dye being spread irregularly throughout the group. In every instance, however, the values of both phenolsulphonephthalein and coefficient are within normal limits.

*Cases Having a Low Coefficient.*—It has been shown that in normal individuals, errors of from 10 to 20 per cent. in the first law of function are to be expected. If, however, in certain groups of cases variations of from 50 to 200 per cent. are of frequent occurrence, and are always in the same direction, one would be justified in assuming that the underlying cause must be some change in renal function.

Four groups of cases have been observed in the present series, which show consistently low coefficients of urea excretion. They are (1) the various fevers, (2) cases of hyperthyroidism, (3) cases of primary hypertensive cardiovascular disease, and (4) cases of early chronic diffuse nephritis, with varying grades of edema and salt retention. In general, they show a normal or somewhat low concentration of urea in the blood, a high rate of urea excretion, a variable concentration of urea in the urine, and a high phenolsulphonephthalein output.

Cases of Fever: Variations of renal function during fever are not unknown. Schlayer<sup>21</sup> mentions fever as one of the extrarenal factors capable of changing renal function. Ambard and Hallion<sup>22</sup> have shown experimentally that the rate of excretion of urea is depressed by lowering the temperature of curarized dogs, without any accompanying change in the level of the blood urea. This fall in output amounted to 12 per cent. for each degree of change. Riste and Kindberg<sup>23</sup> showed a definite lowering of the coefficient in early amyloid disease. They believed this depression was due to the early amyloid changes in the kidney, but, in all probability, a large part of the change was caused by the fever associated with the tuberculous

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21. Schlayer: *Beihefte z. med. Klin.*, 1912, **8**, 211.

22. Ambard and Hallion: *Compt. rend. Soc. de biol.*, 1912, **72**, 931.

23. Riste, Leon, and Kindberg, K.: *Jour. d'urol. med. et chir.*, 1913, **3**, 561.



process. Achard and Leblanc<sup>24</sup> have presented a short series with low coefficient in which are included several cases of fever. They considered the relative increase in the rate of excretion to be a part of the general loss of central control seen in hyperpyrexia.

A considerable variety of cases has been observed, including pneumonia, typhoid fever, acute rheumatic fever, malaria, tuberculosis, pyelophlebitis and acute gout. Observations have been made during both fever and convalescence, and also during intermissions in the fever.

In a majority of cases there was no oliguria; the albumin varied from a heavy cloud to a faint trace, and was completely absent at times. Casts were not constantly present. There was no obvious relationship between the presence or absence of albuminuria and cylindruria and the level of the coefficient.

In Table 2 the various cases are grouped according to the diagnosis, and the arrangement of the subgroups is in the order of ascending coefficients.

The maximum range of the coefficient<sup>25</sup> lies between 0.116 and 0.032, with an average value of 0.051. This is 31 per cent. lower than the average normal coefficient. The coefficient is below 0.06 in 77 per cent. of the cases, and in 55 per cent. is below 0.05, while in the normals no constant was lower than 0.053. These low values of the coefficient are not due to an excessive lowering of the blood urea. The average is 12.7 mg. urea nitrogen per 100 c.c., as compared with the normal of 14.4 mg.

The chief point of interest is that with a slightly lowered blood urea there is associated an enormous increase in the rate of output of urea. This is not associated with a marked change in any *one* of the functions of the kidney. It is not the result of a polyuria, or of any marked increase in the concentrating power; sometimes one condition is present, sometimes the other. While the average rate of urea excretion for a blood urea of 0.308 gm. per liter is 17.2 gm. per twenty-four hours, the average in fever for a similar concentration is 36.4 gm., an increase of more than 100 per cent (Table 3). This cannot be regarded as an evidence of hyperpermeability of the renal epithelium. Any kidney capable of raising the concentration of urinary urea to a level 80 times that in the blood can scarcely be called a hyperpermeable organ. The increased rate of output indicates an increased functional activity of the kidney.

24. Achard and Leblanc: *Presse méd.*, 1914, **22**, 365.

25. The highest coefficient (0.116, Case 414) was obtained in a moribund case. It is probable that cardiac failure was largely responsible for the impaired kidney function. Cloudy swelling was the only change at necropsy.

TABLE 2.—FEVER AND CONVALESCENCE

Date	Case No.	Medical No.	Temperature, F.	Gm. Urea per Liter of Urine, C	Corrected Rate of Output per 24 Hr. $D \times \frac{70}{P} \times \frac{\sqrt{O}}{\sqrt{25}}$	Gm. Urea per Liter of Blood, Ur	Urea Nitrogen, Mg. per 100 C.c. Blood	Coefficient K	Index	'Phthal- ein Output, % 2 Hr.	Diagnosis	Albumin in Urine
1/8/16	400	35269	104.5	17.7	84.1	0.299	14	0.033	533	..	Lobar pneumonia, third day.....	++
1/10/16	...	.....	104.5	26.7	80.2	0.299	14	0.033	533	64	.....	++
1/11/16	...	.....	100.5	30.7	48.7	0.45	21	0.064	156	..	.....	+++
1/12/16	...	.....	99	18.4	44.1	0.407	19	0.061	172	..	.....	++
1/14/16	...	.....	99	32.4	41.6	0.386	18	0.059	184	..	.....	+
1/17/16	...	.....	100	15.3	25.8	0.257	12	0.051	246	73	.....	+
1/22/16	...	.....	99	6.5	13	0.278	13	0.077	108	77	.....	0
1/26/16	...	.....	98.5	10.1	20.5	0.257	12	0.057	197	..	.....	0
3/10/16	401	.....	98.5	31.6	44	0.235	11	0.036	494	..	Lobar pneumonia, day of crisis....	0
3/19/16	...	.....	98	15.7	27	0.15	7	0.029	762	..	.....	0
4/26/15	402	34092	104.5	21.4	31.8	0.235	11	0.042	360	..	Lobar pneumonia.....	F. T.
5/11/15	...	.....	98	25	32.6	0.364	17	0.064	156	..	Lobar pneumonia.....	0
3/18/15	403	33873	103	22.5	42.1	0.235	11	0.045	315	..	Lobar pneumonia.....	+
4/17/15	...	.....	98	13.6	15	0.278	13	0.072	124	..	Lobar pneumonia.....	0
1/17/16	404	35274	103.5	9.2	27.2	0.278	13	0.053	228	70	Lobar pneumonia.....	0
1/19/16	...	.....	103.5	5.6	22.3	0.257	12	0.047	290	..	.....	T
1/20/16	...	.....	99.5	17.2	43.5	0.321	15	0.049	267	..	Crisis.....	+
1/21/16	...	.....	99	32.8	51.5	0.321	15	0.045	315	..	.....	-
1/22/16	...	.....	99.8	28.6	39.4	0.3	14	0.043	278	..	.....	0
1/24/16	...	.....	97	27.1	35.2	0.214	10	0.036	494	74	.....	0



1/26/16	...	....	97	21.4	28.2	0.257	12	0.048	278	..	..	—
1/29/16	...	....	97	5.9	13.8	0.214	10	0.057	197	..	..	—
405	405	94083	100.2	16.7	33.6	0.364	17	0.063	161	..	Lobar pneumonia.....	++
406	406	34794	101.5	11.2	20.4	0.236	14	0.065	152	..	Bronchopneumonia.....	+
407	407	35319	103.5	23.1	57.1	0.493	23	0.065	152	..	Lobar pneumonia.....	++
...	...	....	98.5	15.1	38.8	0.257	12	0.041	380	..	.....	T
408	408	35081	103	17.9	45.6	0.45	21	0.067	143	..	Lobar pneumonia, died 3 days later	+
409	409	34679	104	17.9	65.6	0.257	12	0.032	623	68	Typhoid fever.....	+
...	...	....	100	10.7	16.7	0.214	10	0.052	236	..	Typhoid hematuria.....	+
...	...	....	98	8.6	6.7	0.171	8	0.066	147	59	Typhoid fever.....	T
...	...	....	98.8	6.6	13.4	0.193	9	0.053	228	65	Typhoid fever.....	T
...	...	....	98	5.6	8.7	0.171	8	0.058	190	69	Typhoid fever.....	T
410	410	35159	104	17.6	37.2	0.235	11	0.039	421	82	Typhoid fever.....	0
411	411	35284	104	7	18.4	0.171	8	0.04	400	78	Typhoid fever.....	T
...	...	....	99	4.3	13.9	0.15	7	0.04	400	..	Typhoid fever.....	0
...	...	....	98	8.3	22.9	0.278	13	0.053	190	81	Typhoid fever.....	0
...	...	....	98.2	2.8	8.2	0.107	5	0.037	463	..	Typhoid fever.....	0
412	412	34625	102	9.1	30.2	0.235	11	0.043	346	..	Typhoid fever.....	F. T.
...	...	....	98	6.5	10.9	0.3	14	0.033	74	..	.....	0
413	413	34709	104	8.6	19.7	0.193	9	0.044	331	61	Typhoid fever.....	T
...	...	....	98	3.2	14.6	0.248	12	0.065	152	..	.....	0
414	414	34732	102.6	23.4	61	0.364	17	0.047	290	67	Typhoid fever.....	+
...	...	....	102	20.6	15	0.452	21	0.116	47	..	Typhoid fever, died 3 days later...	++
415	415	34769	104	15.1	26.6	0.272	13	0.053	228	59	Typhoid fever.....	F. T.
416	416	34619	104	7.9	21.2	0.342	16	0.075	114	..	Typhoid fever.....	T
...	...	....	98.5	20.5	20	0.353	17	0.079	102	..	.....	0

TABLE 2.—FEVER AND CONVALESCENCE—(Continued)

Date	Case No.	Medical No.	Temperature, F.	Gm. Urea per Liter of Urine, C	Corrected Rate of Output per 24 Hr. $D \times \frac{70}{P} \times \frac{\sqrt{C}}{\sqrt{25}}$	Gm. Urea per Liter of Blood, Ur	Urea Nitrogen, Mg. per 100 C.c. Blood	Coefficient K	Index	Phthalic Output, % 2 Hr.	Diagnosis	Albumin in Urine
9/15/15	...	....	99	8.5	7.9	0.275	13	0.097	68	50	.....	0
417	34994	....	101.6	9.2	9.7	0.235	11	0.076	111	..	Typhoid fever.....	T
418	35126	....	100	80.9	53.7	0.278	13	0.088	444	72	Tuberculous meningitis, died 3 days later.....	++
12/10/15	...	....	101	26.7	47.5	0.343	16	0.05	256	..	.....	++
419	....	....	102	22.8	30.7	0.257	12	0.046	303	80	Pulmonary tuberculosis; broncho-pneumonia.....	F. T.
11/21/15	...	....	99.8	12.6	19.5	0.214	10	0.049	267	..	.....	T
11/22/15	...	....	100	4.9	12.4	0.321	15	0.005	71	60	.....	T
11/23/15	...	....	100.2	11.7	16.1	0.235	11	0.039	184	68	.....	T
11/25/15	...	....	99.8	3.8	10.5	0.235	11	0.073	120	88	.....	T
12/ 1/15	...	....	.....	8.4	17.8	0.257	12	0.061	172	..	.....	0
12/14/15	...	....	100.5	7.2	14.2	0.214	10	0.057	197	78	.....	0
12/ 3/15	420	....	102	6.2	11	0.214	10	0.064	157	56	Tuberculosis, pleurisy.....	F. T.
12/14/15	...	....	101	21.4	39.8	0.257	12	0.041	380	90	.....	0
12/30/15	...	....	100	8	19.9	0.171	8	0.088	442	68	.....	0
1/26/15	...	....	98	7.7	16.1	0.257	12	0.004	156	83	.....	0
2/27/15	...	....	98	3.8	7.8	0.214	10	0.076	111	61	.....	0
421	....	....	102.5	31.6	35	0.3	14	0.051	246	..	Acute rheumatic fever.....	T
422	....	....	98.6	9.7	11.4	0.171	8	0.051	246	..	Pyelophlebitis.....	0
11/26/15	...	....	103.6	4.7	9.4	0.171	8	0.055	211	..	.....	0
423	....	....	101	....	....	0.214	10	0.047	240	..	Acute gout.....	0
424	35184½	....	96.4	15.5	42.2	0.364	17	0.056	204	..	Malaria.....	0



Associated with this improved nitrogen excretion, there is a well-marked increase in the phenolsulphonephthalein excretion, the average for two hours being 70.5 per cent., compared with a normal of 60.2 per cent.

The cause of this disturbed condition of renal function must be common to all fevers, as there is a complete agreement of the results in all cases. The only features in common are increased temperature and the presence of a toxemia.

The effect of heat on the rate of metabolism has been thoroughly studied. Pflüger<sup>26</sup> found that heating curarized animals caused an increase in the rate of gas exchanges of approximately 10 per cent. for each degree (C.) of rise. Linser and Schmid<sup>27</sup> found increases in the neighborhood of 13 per cent. for each degree of rise, while Voit<sup>28</sup> confirmed these findings by studies on the nitrogen excretion under similar conditions.

TABLE 3.—AVERAGE VALUES OF UREA EXCRETION IN A GROUP COMPRISING HYPERTHYROIDISM, MYXEDEMA AND COLLOID GOITER

Description	Gm. Urea per Liter Blood, Ur	Urea N, Mg. per 100 C.c. Blood	Corrected Rate of Output for 24 Hr. $70 \sqrt{\frac{C}{P}}$ $D \times - \times \frac{1}{\sqrt{25}}$	Rate of Output Calcu- lated for $Ur=0.308$	Coeffi- cient	Index	'Phthal- ein Output, 2 Hr.
Normal.....	0.308	14.3	17.2	17.2	0.074	116	60.2
Fever.....	0.272	12.7	28.6	36.4	0.051	246	70.5
Convalescent.....	0.238	11.1	13.4	22.5	0.065	152	63.2
Hyperthyroid.....	0.233	10.9	19.4	33.8	0.053	228	71.8
Hypertension.....	0.22	10.3	17	33.2	0.053	224	65.7
Chronic diffuse nephritis.	0.21	9.8	13.6	29.2	0.057	197	68.2

In moderate grades of fever, however, the nitrogen excretion frequently increases from 100 to 150 per cent., in spite of a low nitrogen intake. This excessive rise has been regarded as an evidence of the toxic destruction of body protein.

Applying these considerations to renal function, we should expect the cells to share in the generally increased metabolism due to hyperthermia, and the rate of output of urea to increase from 10 to 13 per cent. for each degree of fever.

In these cases, however, the rate of output is frequently doubled, while a simple hyperthermia of 3 degrees (C.) would only account

26. Pflüger: Arch. f. d. ges. Physiol., 1878, **18**, 303, 356.

27. Linser, P., and Schmid, J.: Arch. f. klin. Med., 1904, **79**, 514.

28. Voit: Sitzungsberichte der Gesellschaft für Morphologie u. Physiologie, 1895, **2**, 120, quoted by Lusk: Science of Nutrition, 1909, p. 313.

for a rise of 30 per cent. Again, if the increased rate of excretion were due entirely to an overheating of the cells, the rate of output should vary directly with the temperature. Observations on cases of pneumonia at the time of crisis, and of septicemia with intermittent fever show that there is no mathematical relation between the height of the fever and the rate of excretion. The very moderate changes caused by the hyperthermia are overshadowed completely by some other factor.

The toxins must be regarded, then, as the more important etiologic agents in this increased rate of excretion. It is known that they increase the rate of protein destruction to a marked degree, that is, they stimulate the processes of catabolism. The kidneys also share in this stimulation, and this remarkable increase in the rate of function is the result. This stimulation can go on to outspoken injury of the cell with the production of an acute toxic nephritis. In these cases there is a marked oliguria, with retention of nitrogen and elevation of the coefficient, showing a serious temporary kidney insufficiency. The damage in this instance is due to a destructive action of the toxins, rather than the milder changes which cause irritation only.

The most constant pathologic finding in fever is a cloudy swelling of the organs. Virchow<sup>29</sup> suggests that in some stages, at least, cloudy swelling may be an evidence of hyperactivity and hypernutrition of the cell, rather than of actual degeneration. Schilling's<sup>30</sup> work supports this view. The hyperactivity of the febrile kidney, shown by the above functional studies, would seem to agree with Virchow's suggestion.

All the determinations in the group were made after the temperature had been normal for at least four days.

The averages of the coefficient and phenolsulphonephthalein output show a marked return to normal. Their values are 0.065 and 63 per cent., compared with febrile averages of 0.051 and 70.5 per cent. In spite of this rise, there is a marked fall in the average blood urea, but this is offset by a still greater fall in the rate of output. These findings coincide closely with the accepted ideas of metabolism in convalescence. There is an increase in the anabolic activities, the tissues throw less waste nitrogen into the blood, and the output of urea diminishes.

While the above considerations apply to a majority of the cases, there are some coefficients which do not return to normal. A few of these cases show casts, a persistent trace of albumin, and a high rate of phenolsulphonephthalein excretion. These kidneys have suffered

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29. Virchow: *Reizung u. Reizbarkeit*, 1858, **14**, quoted in Ziegler's *Lehrbuch der allgemeinen Pathologie*, Jena, 1901, p. 206.

30. Schilling: *Virchows Arch. f. path. Anat.*, 1894, **135**, 470.



permanent damage and present in a more or less typical form the functional picture seen in early chronic diffuse nephritis (Group 4).

Cases of Hyperthyroidism: The same marked increase in general metabolism is seen also in exophthalmic goiter. Indeed, Magnus-Levy<sup>31</sup> reports increases in oxygen consumption of from 50 to 70 per cent. over normal figures. Friedrich Müller<sup>32</sup> reports a patient weighing 29 kg., with exophthalmic goiter, who lost weight on a diet containing 68 gm. of protein and 58 calories per kilogram. This points to a greatly increased destruction of protein and fat. The nitrogen excretion frequently rises to very high levels. At times this can be referred to superalimentation, but there is frequently a large negative nitrogen balance. This marked increase in metabolism has been attributed to the tremor present in exophthalmic patients; but calorimetric determinations during sleep have shown that there is no diminution in heat production when there is complete absence of tremor.<sup>31</sup>

With a view to estimating the extent to which the kidney participated in this accelerated metabolism, the coefficient of urea excretion was determined in a number of cases of hyperthyroidism. At the same time two cases of myxedema were studied and one case of colloid goiter. The results are presented in Table 4, and the average values for the group are to be found in Table 3. The cases were outspoken instances of toxic goiter, with varying grades of exophthalmos and tachycardia. In no case was the temperature above 99.5, consequently hyperthermia could not be accepted as an explanation of the findings. A majority of the cases showed tremor, emotional disturbances and loss of weight. Three cases (Cases 459, 461 and 462) showed varying grades of myocardial insufficiency, with chronic passive congestion.

In this series of twelve cases the minimum coefficient is 0.024 (McLean's index 1110), and the maximum 0.079. The average of the 19 determinations is 0.053. The highest coefficient among the cases with outspoken hyperthyroidism, and without cardiac insufficiency, is 0.058. The average blood urea is distinctly lower in this group than in normal cases, but the blood urea and coefficient are absolutely independent of each other. Case 460 shows a blood urea nitrogen of 7 mg. and a coefficient of 0.067, while in Case 450 a blood urea nitrogen of 9 mg. is associated with one of 0.024. Again, Case 461, with a blood urea nitrogen of 13 mg., has a coefficient of 0.047. The consistently low coefficients cannot be related, therefore, to this slight depression of the blood urea, but it is quite possible that the relatively

31. Magnus-Levy and von Noorden: *Metabolism and Practical Medicine*, English translation, 1907, p. 995.

32. Müller, F.: *Deutsch. Arch. f. klin. Med.*, 1893, **51**, 335, 361.

low blood urea may be the result of the increased rate of excretion of urea.

All these cases show a high phenolsulphonephthalein output, the average excretion being 71.8 per cent. in two hours. This is 11.6 per cent. higher than the normal average.

TABLE 4.—HYPERTHYROIDISM

Date	Case No.	Medical No.	Gm. Urea per Liter of Urine, C	Corrected Rate of Output per 24 Hr. $\frac{70 \sqrt{C}}{D \times \frac{P}{V^{25}}}$	Gm. Urea per Liter Blood, Ur	Urea Nitrogen, Mg. per 100 C.c. Blood	Coefficient	Index	Phthal-ein Output, 2 Hr.
	450	.....	....	....	.....	9	0.024	1,110	72
	451	.....	....	....	.....	8	0.036	494	75
	452	35150	12.3	21.9	0.193	9	0.041	381	
12/19/15	453	.....	5.2	13	0.171	8	0.047	290	78
1/22/16	...	.....	12.4	21.3	0.235	11	0.051	246	89
12/18/15	454	S. 38841	10.7	24.4	0.234	11	0.047	290	80
12/19/15	...	.....	6.5	18.9	0.257	12	0.058	190	
1/22/16	...	.....	12.4	22.4	0.257	12	0.054	220	52
	455	.....	....	....	.....	9	0.053	228	64
	456	.....	....	....	.....	11	0.053	228	72
1/24/16	457	S. 38946	22.5	22.5	0.257	12	0.054	220	
2/16/16	...	.....	8.1	11.7	0.235	11	0.058	190	
	458	S. 38280	5.9	9.5	0.171	8	0.055	211	73
	459	.....	9.4	13.2	0.21	10	0.057	197	
	460	S. 38557	5	5.1	0.15	7	0.067	142	
12/13/15	461	.....	10.4	19.8	0.321	15	0.072	124	64
1/24/16	...	.....	15.3	34.7	0.278	13	0.047	290	71
3/ 6/16	...	.....	....	....	.....	14	0.051	247	
	462	.....	....	....	.....	17	0.079	102	
Myxedema									
11/17/15	475	.....	19	7.3	0.257	12	0.097	68	55
12/ 2/15	...	.....	23.7	14.9	0.3	14	0.072	124	65
11/17/15	476	34982	....	....	.....	15	0.125	41	53
11/23/15	...	.....	....	....	.....	22	0.11	53	47

Both these tests show a marked increase in the functional power of the kidney in the uncomplicated cases. The response of the kidney to the stimulus of the blood urea is greater than normal, consequently the rate of output is decidedly higher than usual and the coefficient is depressed. This increased renal activity is probably a local manifestation of the general acceleration of metabolism caused by the hyperactivity of the thyroid gland.



Partial strumectomy, or ligation of vessels, has been done on three of these patients (Cases 454, 457 and 461). One left the hospital unimproved. One (Case 457) showed a distinct increase in glucose tolerance, and some clinical improvement, as evidenced by a gain in weight, but practically no change in the coefficient. He was observed for only three weeks after operation. The third (Case 461) is still in the hospital. She is improving slowly, but so far the coefficient has not shown any marked tendency to return to normal. This slow return to normal after operative interference suggests the possibility that the internal secretion of the thyroid has a definite toxic action resulting in irritation of the kidney tissues. The circumstance that hypertension may develop in thyroid disease<sup>33</sup> also makes it possible that changes have occurred in the smaller vessels throughout the body and that the vascular system of the kidney has suffered at the same time. There is a noteworthy similarity between the above functional findings and those in primary hypertensive vascular disease.

In colloid goiter there is absolutely no change from normal, either in the coefficient or in the phenolsulphonephthalein output.

The findings in two cases of myxedema were particularly interesting. Schwartz and McGill<sup>34</sup> noted a somewhat low concentration of blood urea in a case of myxedema. Two cases in the present studies showed normal concentrations of blood urea (12 and 14 mg. *U-N*). In one case the coefficient was 0.097 and in the other 0.125, while there was a low normal excretion of phenolsulphonephthalein. Both cases showed a marked clinical improvement following the administration of thyroid extract. The blood urea increased to a moderate degree and there was a distinct lowering of the coefficient.

Marshall and Davis<sup>35</sup> have shown that removal of the suprarenals produces a marked impairment of renal function, as measured by the level of blood urea and the phenolsulphonephthalein excretion. This impairment is not due to any failure of circulation, and is undoubtedly caused by the removal of the internal secretion of the glands. The above clinical findings are in close accord with their experimental results, and furnish additional proof of the widespread activities of the glands of internal secretion as regulators of metabolism.

**Primary Hypertensive Cardiovascular Disease:**<sup>36</sup> There were ten cases in this group. All had a systolic pressure above 160 mm. of mercury. In almost every instance the patients complained of some manifestation of the increased blood pressure, such as headaches, transient attacks of vertigo, mild signs of cardiac embarrassment, or

33. Janeway, T. C.: *Bull. Johns Hopkins Hosp.*, 1915, **26**, 341.

34. Schwartz and McGill: *THE ARCHIVES INT. MED.*, 1916, **17**, 42.

35. Marshall and Davis: Personal communication to the author.

36. Janeway, T. C.: *THE ARCHIVES INT. MED.*, 1913, **12**, 755.

vague neurasthenic symptoms. They came for consultation purposes, so that prolonged observations were not possible. Slight cardiac hypertrophy was the only physical finding common to the whole group. The output of urine was somewhat increased, traces of albumin were present in a majority, but casts were found in less than half the cases.

One patient (Case 0, Table 5) has been seen at intervals during eight months. He has shown marked symptomatic improvement and a slight drop in blood pressure, but no change in the urinary picture or in the level of the coefficient.

In these cases the average blood urea nitrogen is 10.3 mg. per 100 c.c., with a range of from 7 to 20 mg. The level of the blood urea is very low in most cases, but in two a high blood urea had no effect on the value of the coefficient. Associated with this

TABLE 5.—PRIMARY HYPERTENSIVE CARDIOVASCULAR DISEASE

Case No.	Medical No.	Gm. Urea per Liter of Urine, C	Corrected Rate of Output per 24 Hr. $70 \frac{\sqrt{C}}{P} \times \frac{D}{\sqrt{25}}$	Gm. Urea per Liter Blood, Ur	Urea Nitrogen, Mg. per 100 C.c. Blood	Coefficient	Index	'Phthal-ein Output, 2 Hr.
0	34088	21.3	15.1	0.193	9	0.05	257	76
		9	13.1	0.171	8	0.048	278	70
10	.....	.....	.....	.....	9	0.05	256	60
14	.....	.....	.....	.....	12	0.051	246	63
1	35486	5.4	14.5	0.193	9	0.051	246	70
5	35552	8.2	14.3	0.214	10	0.055	212	58
6	.....	16.2	17.3	0.235	11	0.056	204	53
12	35239	30.6	50.9	0.406	10	0.057	197	58
7	35477	4.9	6.7	0.15	7	0.058	190	64
18	33777	11.3	10.5	0.193	9	0.059	181	85

depressed blood urea is an increased rate of output. For an average blood urea nitrogen of 10.3 mg. there is an output of 17 gm. per day, or, recalculated for a normal concentration of 14.4 mg., the output is 33.2 gm. This shows an increase of 100 per cent. over the normal response of the kidney to the same concentration of urea in the blood. As a result of this increased rate of output, the coefficient shows a marked depression, the average value being 0.0535. The phenolsulphonephthalein excretion shares in this increased renal function, the average output being 5 or 6 per cent. above the normal average.

According to Fischer,<sup>37</sup> every case of pure hypertension shows a change in the smaller vessels of the body. These vascular changes

37. Fischer: Deutsch. Arch. f. klin. Med., 1913, **109**, 469.



are associated with a loss of vasomotor control, and vascular crises are the result. The same conditions are present in the arterioles of the kidney. There is an increased irritability of the organ, which is shown by an exaggerated response to stimuli of all kinds. The depression of the coefficient is a means of gaging the intensity of this pathologic change. This condition has been described by Schlayer<sup>21</sup> in his various studies of renal function, and the above findings are confirmatory of certain phases of his work.

The primary stage of irritation is of particular value in explaining the outspoken pathologic changes seen in cases of primary contracted kidney in which the functional findings have been normal. These cases are in the second stage of the disease. Having gone through the initial period of irritation (Fig. 1, *A*, *B*, *C*), they have returned to an apparently normal function (*C*), although the damage has progressed. Any further advance in the pathologic process will be shown promptly by functional evidence of renal insufficiency.

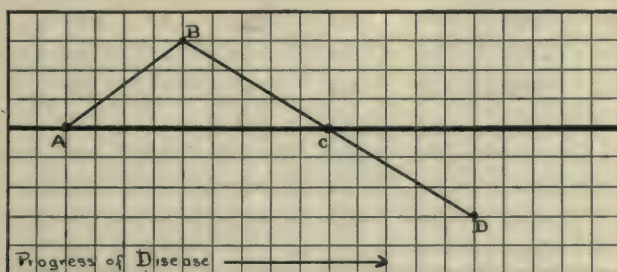


Fig. 1.—State of renal function as shown by progressive renal involvement. *A*, normal; *B*, increased; *C*, "pseudo" normal; *D*, decreased. The heavy horizontal line indicates the normal level. (After Schlayer.)

**Early Chronic Diffuse Nephritis:** Cases of chronic diffuse nephritis showing a hyperpermeability to phenolsulphonephthalein have been described by Sellards,<sup>38</sup> Pepper and Austin,<sup>39</sup> and Baetjer.<sup>40</sup> There were ten similar cases in the present series; a few showed edema at the time of observation and all showed a marked interference with the excretion of sodium chlorid. The urine was highly albuminous (from 3 to 7 per 1,000, Esbach), and casts were frequently present. The specific gravity was high. The nonprotein nitrogen of the blood was low in the four cases reported by Baetjer. We found the same condition in our own series. The blood urea was below normal in every case except one. This patient presented an extreme oliguria during one stage of his illness, and at this time his blood urea rose to

38. Sellards, A. W.: Bull. Johns Hopkins Hosp., 1912, **23**, 298.

39. Pepper and Austin: Am. Jour. Med. Sc., 1913, **145**, 254.

40. Baetjer, W.: THE ARCHIVES INT. MED., 1913, **11**, 593.

triple its former value. The rate of output was reduced in this instance to such a degree that the coefficient rose from 0.05 to 0.214. With the secretion of a larger volume of urine the coefficient promptly fell to its former level. As a rule, however, the rate of output was much greater than that normally found with low concentrations of blood urea and the coefficient was depressed. The average value for the ten cases was 0.057. The phenolsulphonephthalein output was never below 56 per cent. and in a majority of cases exceeded 65 per cent.

TABLE 6.—EARLY CHRONIC NEPHRITIS

Date	Case No.	Medical No.	Gm. Urea per Liter of Urine, C	Corrected Rate of Output per 24 Hr. $D \times \frac{70 \sqrt{C}}{P \sqrt{25}}$	Gm. Urea per Liter Blood, Ur	Urea Nitrogen, Mg. per 100 C.c. Blood	Coefficient	Index	'Phthal-ein Output, 2 Hr.
	11	34529	....	....	.....	10	0.052	237	69
	2	34369	29.4	37.9	0.321	15	0.053	227	65
	3	.....	7.11	18.9	0.235	11	0.054	220	78
1/ 7/16	8	35138	4.6	12.3	0.193	9	0.055	212	71
1/17/16	...	.....	4.6	6.3	0.15	7	0.059	184	
1/18/16	...	.....	14.7	14.5	0.193	9	0.051	246	67
1/19/16	...	.....	3.2	8.9	0.15	7	0.05	256	
	4	35443	19.6	21.3	0.257	12	0.055	212	56
		34679	....	....	.....	5	0.058	190	69
	9	33745	7.3	6.2	0.171	8	0.068	138	70
	11	34529	8.9	9.3	0.257	12	0.07	130	69

In these cases the early inflammatory process has caused a cellular irritation, which is again made evident by an increased response to stimulation. These cases also go through a second stage, in which the usual functional tests are normal, and a later stage, in which the damage is so marked that the functional tests show progressive impairment of renal function.

The last two groups of cases are presented to emphasize the fact that irritation is the earliest sign of renal disease, no matter whether the process be of the vascular or of the diffuse type, and it should be remembered that increased function is one of the first evidences of this irritation. An excessively low blood urea in the presence of a normal protein intake, a coefficient persistently below 0.055, or a phenolsulphonephthalein output of more than 70 per cent. should be regarded, therefore, with suspicion, as being possible indications of an early stage of renal damage.



## CASES WITH INCREASED COEFFICIENTS OF UREA EXCRETION

## Increased coefficients

Myocardial insufficiency; variable, with changes in circulatory system

Nephritis

Constant

Variable

With changes in the clinical picture

Acute nephritis

Progressive renal insufficiency

Effect of myocardial insufficiency

Without changes in the clinical picture

Effect of fixation of specific gravity and interference with water excretion

Basal coefficient

*Myocardial Insufficiency.*—The patients in this group presented some type of pure cardiac disease of a valvular or myocardial nature. Cases of nephritis with failing compensation have been excluded. A number of the patients were followed from the stage of extreme myocardial insufficiency through the period of recovery to a complete restoration of the circulatory equilibrium. Some did not improve during their stay in the hospital. Twelve died; of these, seven came to necropsy. Six of the latter showed typical chronic passive congestion of the kidney. One (Case 276) showed a few arteriosclerotic scars of the kidney, with a marked chronic passive congestion superadded.

*Tests of Renal Function in Chronic Passive Congestion.*—Blood Nitrogen: In chronic passive congestion, the chief functional defect lies in the inability of the kidney to excrete water and sodium chlorid. The concentrating power for nitrogen is rarely interfered with, and little evidence of nitrogen retention in the blood is to be expected, except in the severe cases. The work of Frothingham and Smillie,<sup>41</sup> Tileston and Comfort,<sup>42</sup> Rowntree and Fitz,<sup>43</sup> and other observers confirms this assumption.

The present group, taken as a whole, shows moderate increase in the blood urea. The average urea nitrogen is 19.9 mg. per 100 c.c., compared with the normal average of 14.4 mg. In moderate chronic passive congestion there is no constant change in the level of the blood urea, with improvement in the circulatory condition. Indeed, many of these cases show a rise in the blood urea following a return of cardiac compensation. This is due to the increased protein intake during convalescence.

In some of the more outspoken cases of myocardial insufficiency (Cases 276, 277, 278 and 279) a definite fall in the blood urea accompanies the return of compensation. In no case does this exceed 12 mg.

41. Frothingham and Smillie: THE ARCHIVES INT. MED., 1914, **14**, 541.

42. Tileston and Comfort: THE ARCHIVES INT. MED., 1914, **14**, 620.

43. Rowntree and Fitz: THE ARCHIVES INT. MED., 1913, **11**, 258.

TABLE 7.—MYOCARDIAL INSUFFICIENCY

Date	Case No.	Medical No.	Gm. Urea per Liter of Urine, C	Corrected Rate of Output per 24 Hr. $D \times \frac{C}{\sqrt{25}}$	Gm. Urea per Liter of Blood, Ur	Urea Nitrogen, Mg. per 100 Cc. Blood	Coefficient	Index	'Phthal- ein Out- put, 2 Hr.	Degree of Chronic Passive Conges- tion	Diagnosis
2/ 1/15	250	32383	15.7	19.2	0.235	11	0.054	222	..	0	Aortic insufficiency
3/ 1/15	251	33659	6.4	12.4	0.257	12	0.074	117	51	+	Mitral regurgitation
2/23/15	...	.....	3.6	19.9	0.3	14	0.067	140	..	0	Mitral regurgitation
8/12/15	252	33767	21.2	18.4	0.342	16	0.08	100	46	±	Aortic insufficiency
8/18/15	...	34597	15.2	4.5	0.407	19	0.193	17	..	++	Aortic insufficiency
9/ 3/15	...	.....	12.3	4.5	0.364	17	0.171	22	40	+	Aortic insufficiency
9/11/15	...	.....	14.4	10.6	0.364	17	0.112	51	..	±	Aortic insufficiency
253	...	.....	25	26.3	0.471	22	0.091	77	42	0	Aortic insufficiency
254	253	33893	25.2	27.3	0.396	13	0.081	98	35	±	Myocarditis; auricular fibrillation
255	254	33727	15.4	15.3	0.321	15	0.082	95	..	±	Myocarditis
257	255	33614	12.3	19.2	0.386	18	0.083	33	59	0	Bacterial endocarditis
260	257	34579	13.7	21.4	0.413	16	0.089	80	48	±	Myocarditis
6/19/15	260	34271	4.6	10.8	0.321	15	0.098	67	..	+	Myocarditis
261	...	.....	21.9	27.7	0.314	15	0.06	173	36	0	Myocarditis
5/21/15	261	34723	10.3	10.6	0.321	15	0.098	67	48	+	Myocarditis
5/29/15	262	34687	14.6	17.1	0.406	19	0.099	65	58	±	Mitral stenosis
263	...	.....	27.6	19	0.462	22	0.106	57	..	±	Mitral stenosis
	263	33869	24.1	10.3	0.321	15	0.1	64	..	±	Myocarditis





of urea nitrogen per 100 c.c. One of these cases subsequently came to necropsy, and the kidneys showed a few arteriosclerotic scars. Rowntree and Fitz<sup>43</sup> suggested that this retention might be due to some slight renal change which was not demonstrable under normal conditions, but which became apparent under the extra strain put on the kidneys by the failing circulation.

**Phenolsulphonephthalein Excretion:** In well compensated heart conditions and the milder forms of passive congestion, Rowntree and Fitz<sup>43</sup> have found little interference with the excretion of dye. In outspoken myocardial insufficiency the excretion was much diminished, but returned to normal with restoration of circulatory equilibrium. These observations were confirmed in the present series.

The coefficient of urea excretion has been determined at least once in all cases, and repeatedly in a number of instances.

In well-compensated cardiac disease there is very little change from normal, the average coefficient being 0.082. The highest value under these conditions is 0.103 and the lowest 0.054.

In forty-one determinations there were outspoken evidences of myocardial insufficiency. The average coefficient in these cases is 0.124, with a maximum variation of from 0.074 to 0.248. There is a general relationship between the degree of decompensation and the height of the coefficient in all these cases: the more evident the passive congestion, the higher the coefficient. The most striking agreement, however, is found in those determinations done on the same individual under varying conditions of myocardial sufficiency and insufficiency.

Case 252 is a good example. The diagnosis was syphilis of aorta, aortic insufficiency and myocardial insufficiency. On the patient's first admission, his coefficient was obtained after compensation had been reestablished. It was normal (0.08). The blood urea nitrogen was 16 mg. and the phenolsulphonephthalein excretion 46 per cent. Six months later he returned. He was very edematous and extremely dyspneic. There was marked oliguria, the daily output rarely exceeding 500 c.c. Chronic passive congestion had evidently impaired his renal function, as his power of concentrating urea was markedly diminished. The blood urea had increased slightly, but the coefficient had risen to 0.193, an increment of over 200 per cent. After six days' rest and a restricted fluid intake, there was some clinical improvement, and the blood urea nitrogen dropped to 17 mg. There was no diuresis, the rate of output was essentially the same as on the previous occasion, and the coefficient showed no marked change. In the course of the next two and a half weeks, the patient lost most of his edema, there was a moderate polyuria, and at the time of the third determination, the coefficient had dropped to 0.112. This change was not accompanied by any fall in the blood urea, but was due entirely to the increased rate of urea excretion. The fourth observation was made shortly after the disappearance of myocardial insufficiency. There was a good output of urea, and, in spite of an increased blood urea, the coefficient had returned to normal. The phenolsulphonephthalein excretion was not determined at the height of passive congestion, but subsequent determinations did not show any marked change from the output obtained on the first admission.



Practically every case in which serial determinations were done showed the same relation between the coefficient and the degree of myocardial insufficiency present. In a majority of cases the coefficient shows no relation whatever to the level of the blood urea, indeed a lowering of the coefficient is frequently associated with a rise in the latter value.

Eighteen of the patients had coefficients below 0.12 and four of these died. In thirteen, the coefficient was above 0.12, with nine fatalities. Of the four fatal cases with coefficients below 0.12, two of the patients died of bacterial endocarditis without obvious chronic passive congestion; the other two showed no myocardial insufficiency at the time of observation, and died in subsequent exacerbations. Five of the thirteen fatal cases in the second group showed a temporary improvement, and during this time the coefficient returned to normal values.

The rise in the coefficient should not be regarded as having the same significance in chronic passive congestion as in nephritis. The rise in the latter case is the result of definite anatomic changes in the renal cells. In chronic passive congestion the fundamental cause lies in the failure of the heart to supply a sufficient quantity of blood to the kidneys. This imperfect circulation results in an oliguria. There is a secondary factor, the actual functional deterioration of the cell, due to its impaired blood supply. This prevents the attainment of a requisite degree of concentration to offset the effects of the oliguria. The resultant of these two factors is that the rate of output does not reach the level demanded by the concentration of urea in the blood, and there is a rise in the coefficient. With a return of normal circulatory conditions, the kidney shows a complete recovery of functional activity.

In cardiac disease there is no great change in the level of the total nonprotein nitrogen or urea of the blood. The urea nitrogen forms a somewhat larger proportion of the total nonprotein nitrogen in the more severely decompensated cases than it does normally. The coefficient of urea excretion affords an excellent means of following the effects of passive congestion on renal function. The more severe grades of chronic passive congestion cause marked rises in the constant. Cases with a coefficient about 0.12 usually come to a fatal issue within a relatively short period. In a majority of cases the phenolsulphonephthalein excretion is diminished in marked passive congestion and returns to normal with the reestablishment of normal circulatory conditions.

Nephritis: Ambard,<sup>44</sup> Widal, Weill and Vallery-Radot<sup>45</sup> and McLean<sup>9</sup> have shown that changes in the functional capacity of the

44. Ambard, L.: *Physiologie normale et pathologique des reins*, Paris, 1914.

45. Widal, Weill and Vallery-Radot: *Presse méd.*, 1914, **22**, 565.

diseased kidney can be followed with great exactness by the coefficient of urea excretion. They have found a marked fixation of the coefficient when the disease was stationary, a gradual rise during the progress of the condition, and a fall if the disease showed a tendency to subside. Widal has compared the excretion of urea to the escape of a fluid through an opening of variable size. If it is escaping at a given rate, under a known pressure, it will be necessary to increase that pressure if the size of the orifice is to be diminished without interfering with the rate of output. These laws of hydrostatics hold absolutely within certain wide limits. The same general considerations apply to renal function. Under normal conditions a satisfactory rate of output can be maintained with comparatively low concentrations of urea in the blood. As the amount of healthy kidney tissue is decreased by disease, interference with the rate of urea excretion becomes more outspoken, and a higher concentration of blood urea is required to maintain the original rate of output. The coefficient, therefore, assumes a higher level than previously, but should remain constant at this new value until a further change in renal function occurs. Ambard, however, points out that these conditions prevail only in the presence of a sufficient output of water and that in the oliguric stages of nephritis variations in the coefficient, which cannot be explained by actual changes in the kidney function, are to be expected.

The coefficient is particularly useful in the study of those cases of nephritis which show a retention of nitrogen. In the early stages of chronic diffuse nephritis, with a maximum involvement of the salt excretion, the results are not so dependable. Indeed, there may be an irritation of the kidney, with an actual hyperactivity in the excretion of nitrogen in certain stages, as has been shown previously. In the absence of any marked interference with the chlorid elimination, however, the coefficient may be relied on to indicate an impaired nitrogen excretion in a considerable number of cases, long before any impairment has been shown by a retention of nitrogen in the blood.

There are 162 cases of nephritis in the present series, but the findings will be tabulated in only forty-one cases, which were studied repeatedly. These results will be used for an analysis of varying factors which may cause changes in the value of the coefficient. The whole series will be utilized for the general discussion and observations on prognosis.

**Acute Nephritis:** There were eighteen cases of acute nephritis, seven of which were observed repeatedly. Possible etiologic factors were known in ten instances. Four were associated with purpura, one followed erysipelas, one followed salvarsan therapy, and two were cases of mercury poisoning. In one case there was a history of prolonged exposure to cold and wet.



TABLE 8.—ACUTE NEPHRITIS

Date	Case No.	Medical No.	Total Non-protein Nitrogen, Mg. per 100 C.c. Blood	Urea Nitrogen, Mg. per 100 C.c. Blood	Percentage of Total Non-protein as Urea Nitrogen	Coefficient	Index	'Phthal-ein Output, 2 Hr.
4/10/15	....	.....	..	34	....	0.116	...	40
4/13/16	....	.....	..	16	....	0.062	...	
4/16/16	....	.....	..	11	....	0.069	...	55
4/18/14	160	20990	..	45	....	0.13	88	*
5/10/14	....	.....	..	39	....	0.104	59	
5/ 2/14	162	20948	..	44	....	0.134	86	15
5/30/14	....	.....	..	22	....	0.079	108	50
2/19/15	85	83695	44	27	61.5	0.172	21	40†
3/ 1/15	....	.....	20	10	50	0.063	163	65
10/23/15	95	84901	60	46	76.7	0.232	12	
10/26/15	....	.....	60	45	75	0.208	16	48
11/ 1/15	....	.....	57	36	63.2	0.162	24	
11/ 3/15	....	.....	49	27	55.1	0.124	42	
11/ 9/15	....	.....	..	24	....	0.107	56	44
11/16/15	....	.....	..	22	....	0.119	45	
11/30/15	....	.....	30	22	73.3	0.111	52	50
12/ 6/15	....	.....	19	12	63.2	0.074	117	
12/ 8/15	....	.....	14	9	64.3	0.054	219	58
12/13/15	....	.....	16	9	56.1	0.061	172	63
12/23/15	132	S. 36610	129	117	90.8	0.4	4	15
12/26/15	....	.....	..	45	....	0.179	3	23
9/29/15	119	84753	178	148	83.2	1.09	0.5	‡
9/30/15	....	.....	170	141	83	1.36	0.3	T.
10/ 2/15	....	.....	125	102	81.6	0.55	2.1	11
10/ 4/15	....	.....	78	55	70.5	0.41	3.8	
10/ 6/15	....	.....	42	23	54.8	0.186	18	27
10/ 8/15	....	.....	26	11	42.4	0.124	42	35
10/13/15	....	.....	22	8	36.4	0.064	156	45
10/18/15	....	.....	22	11	50	0.12	53	

\* The diagnosis was acute nephritis; purpura.

† The diagnosis was salvarsan poisoning.

‡ The diagnosis was mercury bichlorid poisoning.

The results were similar in every case. During the acute stage there was a variable grade of oliguria and a well-marked retention of nitrogen, as shown by studies of the blood. The coefficient showed a rise corresponding to the severity and duration of the disease, and there was a consistent diminution in the phenolsulphonaphthalein output. The highest values were found in Cases 95, 119, and 132. In Case 95 the nephritis was associated with a septicemia, and was probably of a focal nature. The second was a case of mercury bichlorid poisoning. The patient had been anuric for six days before examinations were begun. Case 132 was of an acute nephritis, with complete suppression of urine for two days after the onset. The two determinations were made during the period of recovery, but the coefficient was not obtained after complete recovery.

In every case clinical improvement was accompanied by a rapid change in the functional tests. The blood urea fell to normal levels, the rate of excretion of urea increased and there was a marked drop in the coefficient. The phenolsulphonaphthalein excretion also increased and resumed a normal rate of output. Those cases in which a chronic nephritis followed the subsidence of the acute lesion gave evidence of this in the persistence of some elevation of the constant and an impaired phenolsulphonaphthalein excretion. In those patients with perfect recovery the coefficient and phenolsulphonaphthalein resumed absolutely normal figures.

**Chronic Nephritis:** The findings in chronic nephritis have been confirmatory in nature to a considerable degree. Ambard and McLean have both noted marked increases in the coefficient, corresponding to the grade of renal insufficiency present.

Reference to the work of Folin,<sup>46</sup> Frothingham,<sup>41</sup> Tileston<sup>42</sup> and others quoted previously shows the wide range of normal values of urea nitrogen. The upper limit of normal is between 20 and 25 mg. Of sixty-six cases in the earlier stages of nephritis, only twenty-two showed urea nitrogens above 20 mg., and only six were above 25 mg.; yet all had coefficients above 0.09 (the upper normal limit), and many showed outspoken increases in the constant. In this same group the urea nitrogen was 15 mg. or less in twenty-nine cases. It is evident from this that the coefficient picks out instances of early impairment long before the blood urea shows any definite increase. In the severer cases there was a more evident agreement between the blood urea and the level of the coefficient. Of forty-one determinations on cases having a coefficient between 0.15 and 0.3, the blood urea was above 25 mg. in thirty-three and above 20 mg. in thirty-seven instances, with only two cases below 15 mg. Both these patients, Cases 88 and 92, were

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46. Folin, O., and Denis, W.: Footnotes 16 and 19.



on a low protein diet at the time, their levels of blood urea nitrogen having been 19 and 65 mg., respectively, before the diet was commenced. In one instance, Case 92, the change in level of the blood urea had caused no noticeable change in the value of the constant, while in Case 88 the disappearance of a moderate passive congestion readily explained the lowered coefficient.

**The Relation of the Total Nonprotein Nitrogen to the Urea Nitrogen:** In a considerable number of cases the total nonprotein nitrogen was determined synchronously with the blood urea nitrogen. Folin and Denis<sup>46</sup> found that 50 per cent. of the total nonprotein nitrogen was present as urea nitrogen in healthy adults, but that in normal clinical material the proportion ranged from 41 to 74 per cent. While this range of variation has been noted in the milder cases of nephritis, an almost complete disappearance of the lower values has been found in the more severe cases. In thirty-eight determinations on persons having a coefficient between 0.09 and 0.15, twenty-one had less than 60 per cent. of the total nonprotein nitrogen as urea nitrogen; while in seventy-one determinations on cases with a constant above 0.15, only eleven had less than 60 per cent. of the total nonprotein nitrogen as urea nitrogen. The ratio of total nonprotein nitrogen to urea nitrogen seems to have a much more intimate relation with the degree of nitrogen retention than it does with the level of the coefficient. The lower percentages are almost always associated with the low values of blood nitrogen. In those cases in which the blood nitrogen was varied by dietary procedure (Cases 117, 88, 92, 54 and 35), the fall in the blood urea always took place much more rapidly than did that of the total nonprotein nitrogen, with the result that even in severe cases the proportion frequently sank below 50 per cent., and went to 43 per cent. on two occasions. In chronic nephritis a low percentage of urea nitrogen has little significance. Percentages above 75 are seen rarely in the milder cases, but they become increasingly frequent in the severer ones.

**The Independence of the Coefficient and the Blood Urea:** If Addis and Watanabe's<sup>10</sup> criticisms of the coefficient are founded on fact, and if the coefficient really maintains its constancy only by reason of the relative fixation of the blood urea, one would be justified in making the following assumption:

1. In cases of chronic nephritis, in a stationary condition, the coefficient should follow the concentration of urea in the blood. Every rise or fall in the blood urea should be reflected by a corresponding variation in the coefficient.
2. If the clinical condition of the patient is showing marked variations during the period of observation, then the variations in the blood

urea, and consequently the coefficient, should reflect the functional condition with accuracy.

3. If the disease should be rapidly advancing, and yet the blood urea should be reduced by dietary measures, then the coefficient should follow the blood urea, and indicate an apparent improvement in the presence of an outspokenly progressive impairment of renal function.

In dealing with the first assumption, reference may be made to Cases 92, 97, 110 and 84. In Case 92 (Fig. 2) variations in the blood urea nitrogen from 59 to 15 mg. per 100 c.c. produced a maximum variation in the constant of from 0.224 to 0.292. In this case the rate of output, corrected for urinary concentration, amounted to 29.7 gm.

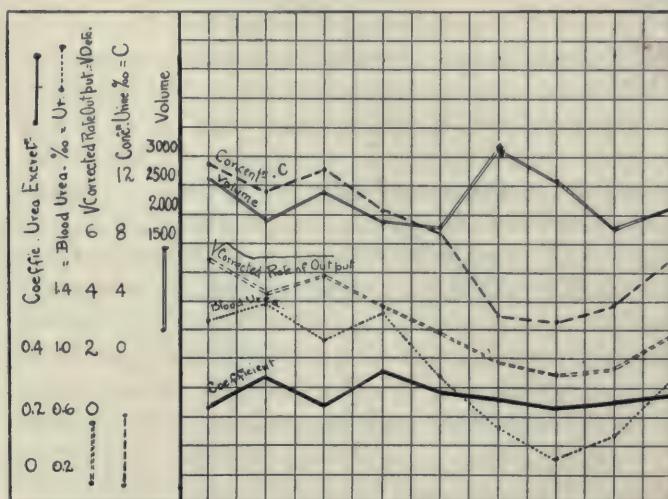


Fig. 2.—Independence of the coefficient of blood urea excretion in chronic nephritis. The coefficient remains constant, while the blood urea and the urea output vary. In this and the following illustrations the full, dark line indicates the coefficient of urea excretion ( $K$ ), the figures being given in the first column at the left; the dotted line indicates the blood urea in thousandths ( $Ur$ ), the figures being given in the second column from the left; the parallel dash line indicates the  $\sqrt{V}$  corrected rate of output  $= \sqrt{V D}$ , etc., the figures being given in the third column; the dash line indicates the concentration of the urine in thousandths ( $C$ ), the figures being given in the fourth column; and the parallel lines indicate the volume of urine, expressed in the figures of the fifth column.

of urea for the initial concentration in the blood. With the drop in the blood urea to 25 per cent. of its former value, the rate of output dropped to 2.09 gm. in the 24 hours, or 7.01 per cent. of its original rate. The other cases all show the same absolute relationship between the rate of excretion and the concentration of urea in the blood. Judged from the blood urea alone, these patients should be considered



as markedly improved, while in reality their functional power has shown no obvious change.

The phenolsulphonephthalein excretion in these cases was constant, pointing to an unchanged condition of renal function.

The acute nephritides furnish the best examples of the agreement of the blood urea, the coefficient, and the clinical condition of the patient. Such cases cannot be used as arguments against the validity of the coefficient.

The third condition is somewhat difficult of attainment, but Case 35 fulfils all requirements.

The patient, C. C. (Med. No. 35242), 35 years of age, was perfectly well until nine months before admission, when headaches began. They became progressively worse. He had marked nycturia; gastro-intestinal disturbances appeared, and he lost a great deal of weight. Visual disturbances commenced one week before admission.

Physical examination showed evident loss of weight, no marked pallor, some exophthalmos, lungs with few râles at the bases. The region of cardiac dullness was 4 by 14 cm. There was relative mitral insufficiency. The blood pressure was 240 systolic, 140 diastolic. There was marked arterial thickening. The liver edge was palpable 2 cm. below the costal margin. The eyegrounds showed extreme hemorrhagic albuminuric retinitis. The urine specific gravity was 1.015. There was albumin, 2 parts per thousand, with numerous hyaline and granular casts. The phenolsulphonephthalein excretion in two hours was 40 per cent.

A diagnosis was made of chronic diffuse nephritis, secondary contracted kidney, chronic uremia, hypertension and arteriosclerosis.

On account of severe headache, he was put on low protein diet. The blood urea nitrogen dropped from 17 mg. to 13 mg. and then to 7 mg. With this drop there was an even greater rate of fall in the twenty-four hour output of urea, and as a result, the coefficient rose from 0.103 to 0.129. The phenolsulphonephthalein excretion dropped to 34 per cent. in the course of a few days.

Under treatment his headaches decreased, but otherwise his condition was unchanged. He died two months later in typical uremic convulsions. In this particular instance the coefficient and phenolsulphonephthalein tests showed the true progressive character of the disease, in spite of the drop in the blood urea and the symptomatic improvement.

From the cases given above it is evident that there can be no absolute relationship between the blood urea and the coefficient. In the mild and moderately severe cases the value of the coefficient depends on the functional capacity of the kidney, and any variation in the value has its underlying cause in some functional change in the kidney itself.

**Causes of Variation in the Coefficient in Chronic Nephritis:** The foregoing cases show conclusively that simple variations in the blood urea have no effect on the level of the coefficient. Therefore, the

most obvious cause of the variability in the constant will be an actual change in the patient's condition. This may be due to changes in circulatory mechanism or to definite changes in renal function.

In myocardial insufficiency the onset of passive congestion is rapidly followed by a rise in the coefficient, which falls again quite as rapidly with the subsidence of the condition. Case 28 shows the effect of an increasing grade of cardiac insufficiency, a marked increase in the coefficient, with very slight change in the blood urea, while Case 52 is an example of a change in the opposite direction. Case 88 shows the effect of chronic passive congestion on the coefficient in a severe case of nephritis. The coefficient falls from 0.192 to 0.154 with an improvement of the circulatory condition, then, as chronic passive congestion returns, rises again to 0.172. In this particular instance the level of the blood urea also shows large variations, due to the dietary regimen. This patient died of cardiac failure two weeks later.

**Actual Changes in Renal Function—Improvement:** The fall of the coefficient during improvement has been discussed under acute nephritis.

By dietary methods, having as their basis a prolonged reduction in the protein intake, we have been able repeatedly to reduce the blood urea to normal and even low normal values, but in only one case has this been followed by any apparent functional change in the kidney. In Case 84, after a low protein intake of two months' duration, the coefficient fell from 0.168 to 0.066. It subsequently rose to 0.087. That this variation was not due merely to a change in the blood urea concentration is shown by the fact that on one occasion the coefficient was 0.163, with a blood urea nitrogen of 12 mg., and one month later was 0.087, with blood urea nitrogen of 13 mg. This patient had been admitted five times during the preceding three years, and on each occasion his phenolsulphonephthalein excretion had been approximately 40 per cent. On the last admission his output was 31 per cent., but with the falling Ambard, his phenolsulphonephthalein rose to 63 per cent. and finally 72 per cent. In other words, there seemed to be a definite functional improvement in this case, which, to the best of our knowledge, had been in a stationary condition for the preceding thirty-six months.

**Advance in the Severity of the Disease:** The coefficient affords an excellent means of studying the progress of functional impairment. Table 9 presents numerous examples of a rising coefficient, associated with increasing renal insufficiency. In these cases an increase in the level of blood urea usually accompanies the rising constant. If such a condition occurred with a stationary lesion of the kidney, the rate of output would increase to a corresponding degree and the coefficient would not change. A rise in the coefficient indicates an inadequate



response on the part of the kidney to an increased stimulation in the form of increased concentration of blood urea. According to the stage of disease, the increase may be slow or rapid. In Case 164 the coefficient rose from 0.172 to 1.12 in six weeks, and death occurred shortly after the latter determination. In Cases 116 and 114 the change was equally rapid.

Other cases have advanced much more slowly. Case 96 presented a coefficient of 0.24 in 1915. Eight months later the value was 0.34 and one year later 0.414. The phenolsulphonephthalein output fell from 10 per cent. to 3.5 per cent., and finally to zero in the same time. Another case (Case 124) merely advanced from 0.101 to 0.153 in three months, while others (Case 92, etc.) were absolutely stationary.

**Changes in the Coefficient Which Are Not Due to Actual Changes in Renal Function:** These changes are only seen when kidney function is in an extremely embarrassed condition.

Certain cases of marked renal insufficiency in the present series showed marked changes in the value of the coefficient, quite independent of any change in the functional efficiency of the organ. Some explanation of these findings was sought in view of their apparent disagreement with the laws of function.

The use of test meals for renal function has shown that the most constant qualitative finding in moderately severe nephritis is loss of concentrating power.<sup>3</sup> This eventually progresses until there is a loss of diluting power as well<sup>5</sup> and the concentration of the urine becomes practically constant throughout the day. Under these conditions the second law of function becomes nonexistent, and the rate of output varies directly as the square of the concentration of urea in the blood. The only means of changing the rate of output is by a variation of the urinary volume, and in such persons a rise in the blood nitrogen is met by a corresponding grade of polyuria. Cases 110 and 112 are examples of this condition. In both cases there is approximate fixation of the urea concentration. In the former case the blood urea drops, and in the latter there is a considerable rise. In each instance the burden of changing the rate of output falls on the volume of urine excreted. In the first case there is an almost perfect response, with a constant coefficient; in the latter (Case 112) the rise in volume is not quite sufficient and there is a definite increase in the constant.

In the later stages of nephritis, even this water-regulating mechanism may be lost, and the rate of output becomes a relatively fixed quantity. As a result the formula

$$K = \frac{\text{Blood urea}}{\sqrt{\text{Corrected rate of output}}} \text{ becomes } K = \frac{\text{Blood urea}}{\sqrt{\text{Constant factor}}}$$

or, in other words, the coefficient varies directly with the blood urea,

because the rate of output has become a constant. The best example of this condition is Case 102 (Fig. 3).

Another possible condition would be the inadequacy of the water output. The frequency with which a diurnal oliguria and nocturnal polyuria are associated in nephritis is well known. If, therefore, the concentration were fixed, and a coefficient were determined during the period of nocturnal polyuria, and again during the oliguria period of the day, the coefficient should vary in direct proportion to the water output. The last four determinations of the coefficient in a case of polycystic kidney (Case 111, Fig. 4) show this condition remarkably well. The blood urea is relatively constant, while the volume, corrected rate of output, and value of the coefficient agree absolutely. The final intense grade of oliguria was precipitated by the ingestion of an enormous quantity of water, given in the hope of producing a polyuria. This is a good example of a fatigue reaction in a badly damaged kidney and is of the same type as that produced in many cases of chronic diffuse nephritis by a sudden overload of salt.

In this case the changes in the value of the coefficient were not associated with any clinical change in the patient. At first sight they seem to be obvious proof of the inaccuracy of the coefficient and the laws of excretion. Closer examination, however, proves them to be the natural outcome of the functional conditions present.

In patients with a regular advance in the kidney lesion, the importance of oliguria in producing the terminal renal insufficiency is beautifully shown in Figure 5. The fixation of concentration is almost complete, although it shows a slight tendency to rise with the progressive increase in the blood urea. The volume and rate of output continually diminish, and finally there is the onset of the premortem protein catabolism, with a tremendous rise in the coefficient and blood urea, associated with a marked fall in the urine volume and rate of excretion. This progressive impairment is also reflected by the constant fall in the phenolsulphonephthalein output.

**Basal Coefficient:** In dealing with physiologic problems we are accustomed to speak of stimuli as being minimal, optimal and maximal. By the last is meant a stimulus of such a strength that it will elicit the maximal response from the tissue under examination. Any further increase in the stimulus will cause no increased response; on the contrary, it may produce actual damage and the response be diminished rather than augmented.

It is conceivable that similar conditions might exist in cases of nephritis with a marked impairment of concentrating power. Within certain limits of blood urea concentration, the kidney would be capable of excreting the theoretical quantities of urea, and the coefficient would remain constant. If the blood urea rose above these limits, and pre-



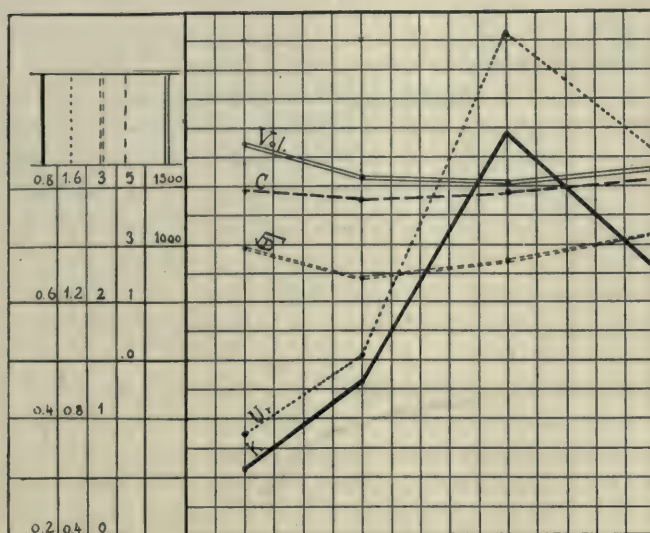


Fig. 3.—The coefficient of urea excretion varies directly with the blood urea in the later stages of nephritis. For key to the diagram see the legend of Figure 2.

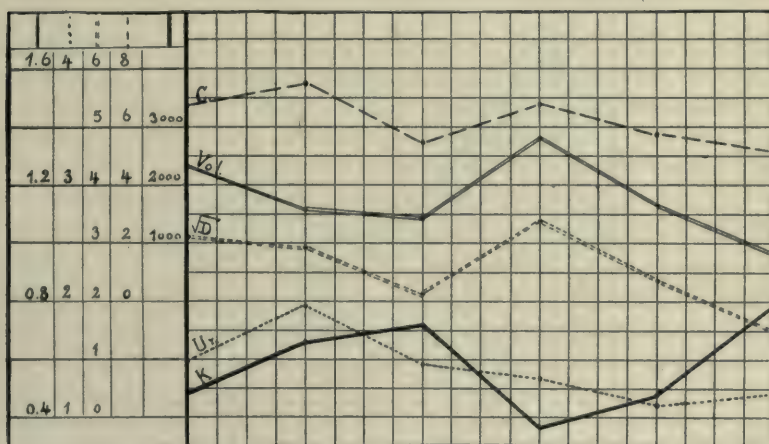


Fig. 4.—Case of variation of the coefficient of urea excretion with changes in the urine volume. For key to the diagram see the legend of Figure 2.

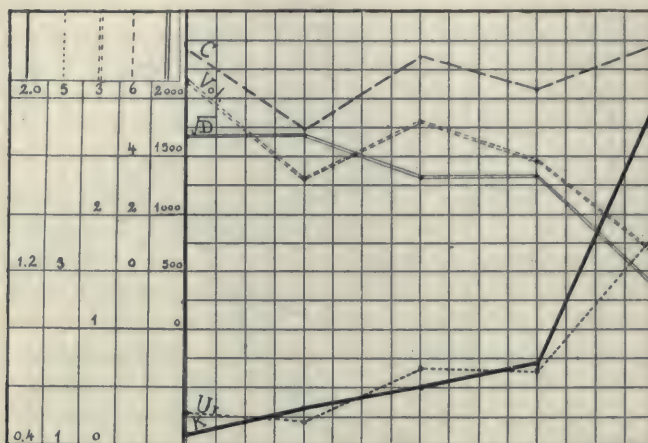


Fig. 5.—Importance of oliguria in producing the terminal rise of the coefficient. For key to the diagram see legend of Figure 2.

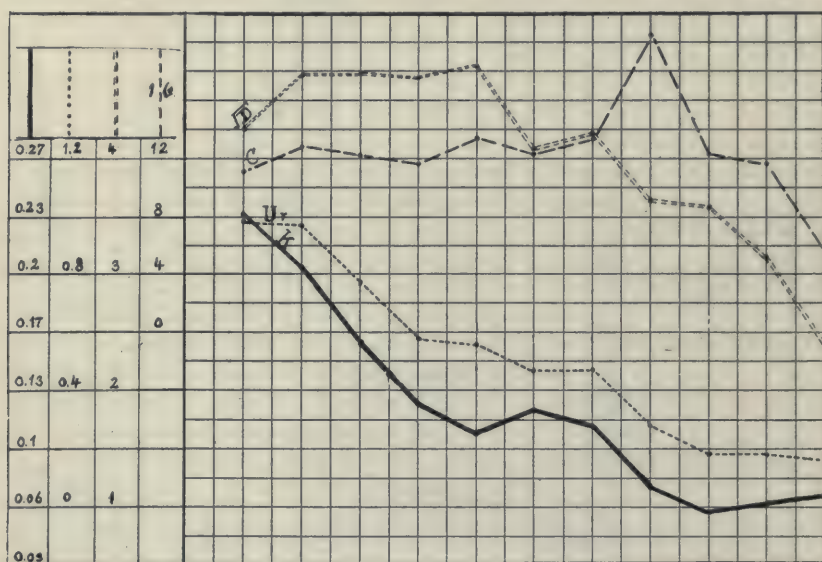


Fig. 6.—Case of direct variation of the coefficient of urea excretion with the blood urea. The rate of urea output remains constant while the urea in the blood is high; when the latter falls, the rate of output begins to vary in relation to the blood urea. For key to the diagram see legend of Figure 2.



sented a supramaximal stimulus, the rate of output would not increase to a corresponding degree, but would remain a relatively fixed quantity. Under these conditions the coefficient would vary directly with the blood urea. The reappearance of a constant coefficient would signify the return of the blood urea within the foregoing limits of concentration and below the critical concentration.

Case 95 (Fig. 6) is of interest in this connection. During the acute stage there was a considerable nitrogen retention. In the first five determinations, however, there was a progressive fall, both in the blood urea and in the coefficient. During this period the rate of output remained practically constant, a good example of a maximal response to a supramaximal stimulus. As soon as the blood urea fell below the critical concentration, the rate of output began to vary in relation to the blood urea.

Case 117 (Fig. 7) is another example of the same condition in a chronic nephritis with the diffuse type. There was marked albuminuria and cylindruria. The specific gravity was fixed in the neighborhood of from 1.010 to 1.012. On admission, the blood urea nitrogen of this patient was 53 mg. per 100 c.c., and the coefficient 0.75. After a period of improvement his protein intake was increased and the blood urea nitrogen rose to 88 mg. The coefficient, however, fell to 0.458. One month later the coefficient was 0.425. He then became worse; there was a marked retention of nitrogen, and the coefficient rose to 1.305. A low protein dietary reduced the level of the blood urea. The coefficient followed it closely during the first part of its fall, but when the coefficient reached the value of 0.525, it became constant. The concentration of urea continued to fall, but each diminution was accompanied by a corresponding change in the rate of output, so that the coefficient resumed its independence of the blood urea concentration. A subsequent rise of the blood urea nitrogen to 64 mg. was associated with a prompt rise in the constant, while subsequent falls below 50 mg. did not produce any marked depression in the coefficient. As the fatal issue approached, renal function became more and more impaired and there was a progressive increase in both blood urea and the coefficient.

After the initial exacerbation of the disease, the phenolsulphonephthalein excretion remained constantly between 5 per cent. and 7 per cent., and showed no such variations as those seen in the coefficient during the same period.

The second case is of particular interest. It raises the question as to whether two types of nitrogen retention should not be recognized. The first type is characterized by a fairly rigid adherence to the laws of function, and every change in blood urea concentration is accompanied by a corresponding change in the rate of excretion. The retention may be regarded as a purely physiologic means of over-

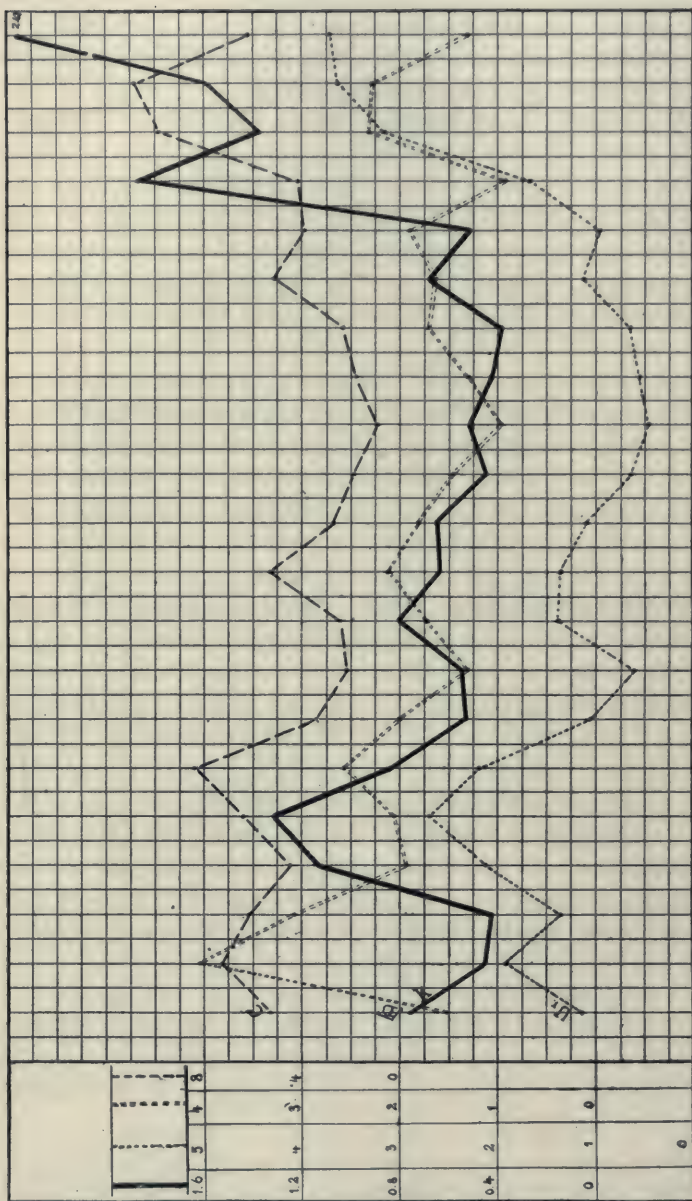


Fig. 7.—Variation of the coefficient of blood urea excretion with the blood urea in a chronic nephritis of diffuse type. For key to the diagram see legend of Figure 2.



coming the increased resistance to the passage of urea through the damaged kidney. The result of such a retention is a coefficient somewhat above the normal, but constant in value. In the second type of retention the conditions are somewhat different. If the kidney were stimulated to its maximal capacity by that portion of the retained urea necessary to produce a maximal response, then the excess above this level would act merely as an overload. It would not have any favorable effect on the rate of urea excretion, and would be of a distinctly pathologic nature. It should be regarded as an accumulation of nitrogen, rather than a retention. Under such conditions the value of  $K$  would vary directly with the concentration of the blood urea. If, however, the blood urea fell below the critical concentration, which marked the division between the pathologic accumulation and the physiologic retention of nitrogen, the kidney would again be able to respond to variations in the blood urea by corresponding variations in the rate of output, and the coefficient would again become a constant. The coefficient would be independent of the concentration of urea in the blood just so long as the blood urea remained below the critical concentration. Reference to Case 117, Table 9, will show that before the exacerbations in October the kidney had quite a wide margin of safety. Concentrations of blood urea nitrogen up to 88 mg. were not sufficient to cause any variations in the coefficient. At a later period 50 mg. seemed to be the value and any rise above the level caused marked rise in the coefficient. It seems justifiable to assume that no absolute change in the functional capacity of the kidney took place during this time, but that the variations in the coefficient were merely the result of this *accumulation* of nitrogen in the blood.

The term "basal coefficient" might be applied to the value of the coefficient below which it is impossible to go, no matter how low may be the value of the blood urea. It represents the actual functional power of the kidney more accurately than do the higher values of the coefficient obtained during the period of nitrogen accumulation.

McLean<sup>47</sup> has described an accumulation of nitrogen in certain cases of nephritis, caused by the limitation of the fluid intake. In the present instance there was no restriction of fluids and the condition was produced by the actual inability of the kidney to excrete either water or urea.

#### PROGNOSIS

While tests of function should be regarded as valuable aids to an accurate prognosis, they should never be allowed to dominate the picture. A diagnosis of the type of lesion and a proper valuation of the many factors in the disease complex are of even greater importance.

47. McLean, F. C.: Jour. Exper. Med., 1915, **22**, 366.

TABLE 9.—CHRONIC NEPHRITIS

Date	Case No.	Medical No.	Total Non-protein Nitrogen, Mg. per 100 C.c. Blood	Urea Nitrogen, Mg. per 100 C.c. Blood	Percent- age of Total Non- protein Nitrogen Present as Urea Nitrogen	Gm. Urea per Liter of Urine, C	Corrected Rate of Output per 24 Hr. $D \times \frac{70}{P} \times \frac{\sqrt{C}}{\sqrt{C_0}}$	Gm. Urea per Liter of Blood, Ur	Coeffi- cient	Index	Phthal- ein Output, 2 Hr.	Diagnosis
3/10/15	23*	33336	28	17	60.8	15.4	14.9	0.364	0.094	72	50	Chronic diffuse nephritis; secondary contracted kidney
3/17/15	...	.....	35	21	60	18.6	24.6	0.45	0.091	78	..	
3/ 1/15	24	33786	25	15	60	13.5	10.7	0.321	0.097	70	35	Primary contracted kidney; myo- cardial insufficiency
4/25/15	...	.....	..	14	....	4.1	5.6	0.209	0.126	40	32	
5/20/15	26*	33405	33	25	75.8	....	....	0.535	0.098	67	41	Chronic diffuse nephritis; secondary contracted kidney
6/22/15	28	34208	30	15	50	9.1	10.7	0.321	0.098	67	42	Chronic diffuse nephritis; secondary contracted kidney
12/ 2/15	35*	35242	..	17	....	7.7	8.7	0.364	0.133	42	57	
12/ 7/15	...	.....	25	17	65.5	12.1	12.5	0.364	0.103	60	40	Chronic diffuse nephritis; secondary contracted kidney; uremia
12/13/15	...	.....	23	13	56.5	4.9	6.9	0.278	0.106	57	34	
1/21/16	...	.....	16	7	43.7	4	1.3	0.15	0.139	38	..	
2/ 8/15	39	33557	..	19	....	14.8	8.7	0.406	0.137	34	32	
2/23/15	...	.....	50	33	66	19.3	45.1	0.706	0.105	58	55	Chronic diffuse nephritis
3/ 5/15	...	.....	30	20	66.6	12.7	25	0.428	0.085	89	..	
3/17/15	...	.....	29	18	62	23.4	40.7	0.385	0.061	175	..	
3/20/15	41*	33821	26	15	57.7	13.6	38.2	0.322	0.053	228	65	Arteriosclerosis; arteriosclerotic kidney contracted kidney; myo- cardial insufficiency
3/20/15	52	33887	23	17	60.5	6.1	9.3	0.364	0.112	53	55	
			20	12	60	8.9	4.9	0.257	0.116	48	66	



4/ 6/15	...	.....	22	13	59.1	11.9	9.6	0.278	0.097	70	..	Primary contracted kidney; no myocardial insufficiency
8/28/15	54*	34404	..	24	....	7.1	18.5	0.514	0.118	46	35	Chronic diffuse nephritis; secondary contracted kidney
12/ 3/15	...	35075	..	16	....	1.2	1.1	0.344	0.331	5.8	24	Chronic diffuse nephritis; secondary contracted kidney; uremia
1/ 8/16	...	.....	108	86	79.5	6.3	9.7	1.84	0.59	1.8	11	
1/14/16	...	.....	73	50	68.5	3.3	3.6	1.07	0.57	1.9	14	
1/24/16	...	.....	41	27	66	2.1	1.9	0.579	0.41	3.9	14	
4/ 3/15	65	34005	25	15	60	11.9	5.8	0.321	0.134	36	15(?)	Primary contracted kidney
4/27/15	...	.....	36	27	75	21.2	14.3	0.578	0.158	26	34	
6/19/15	...	.....	..	27	....	19.2	16.4	0.578	0.142	32	20	
4/29/15	70*	34100	39	34	87.3	19.4	26.9	0.729	0.14	33	25	Chronic diffuse nephritis
5/21/15	...	.....	111	88	79.2	22.2	32.0	1.584	0.334	5.7	T	Chronic diffuse nephritis; uremia
1/17/16	71*	35182	32	17	53.1	19.7	6.6	0.364	0.141	32	55	Primary contracted kidney; cerebral arteriosclerosis
3/18/16	74*	35264	..	10	....	5.8	2.2	0.214	0.144	31	34	Primary contracted kidney; chronic diffuse nephritis
5/24/15	84	34239	35	28	89	15.4	12.7	0.6	0.775	1	0	Primary contracted kidney; chronic diffuse nephritis; uremia
8/12/15	...	.....	..	61	....	20	65.8	1.305	0.161	23	45	Chronic diffuse nephritis
11/13/15	...	.....	..	23	....	7.9	1.9	0.483	0.361	25	37	
11/16/15	...	.....	..	26	....	16.2	16.1	0.557	0.139	5	..	
11/29/15	...	.....	24	12	50	10.3	7.3	0.257	0.095	33	31	
12/ 8/15	...	.....	14	5	35.7	2.4	2.6	0.107	0.066	71	54	
12/20/15	...	.....	18	8	44.5	3.6	4.6	0.171	0.06	144	63	
12/30/15	...	.....	..	13	....	13.8	10	0.274	0.08	100	72	
4/28/14	164*†	26982	..	42	....	....	27.6	0.904	0.087	84	70	Chronic diffuse nephritis; secondary contracted kidney
5/25/14	...	.....	..	77	....	....	27.8	1.65	0.172	22	19	
									0.312	6.6	..	

\* Cases ending fatally.

† Cases in which original hypobromite methods were used for analysis.

TABLE 9.—CHRONIC NEPHRITIS—(Continued)

Date	Case No.	Medical No.	Total Non-protein Nitrogen, Mg. per 100 C.c. Blood	Urea Nitrogen, Mg. per 100 C.c. Blood	Percentage of Total Non-protein Nitrogen Present as Urea Nitrogen	Gm. Urea per Liter of Urine, C	Corrected Rate of Output per 24 Hr. $\frac{D \times -x - \frac{P}{\sqrt{25}}}{70 \frac{V}{C}}$	Gm. Urea per Liter of Blood, Ur	Coefficient	Index	'Phthal- ein Output, 2 Hr.	Diagnosis
6/ 6/14	...	.....	..	178	....	....	11.4	3.769	1.12	0.5	0	Ch. dif. nephritis; secondary cont'd kidney; myocardial insufficiency
1/12/16	88*	35287	23	19	82.5	8.3	4.5	0.407	0.192	17.4	24	
1/28/16	...	.....	24	12	50	6.3	2.8	0.257	0.154	27	23	
2/24/16	...	.....	30	26	66.6	10.6	10.5	0.556	0.172	22	..	Chronic diffuse nephritis; secondary contracted kidney Chronic diffuse nephritis; secondary contracted kidney
3/ 8/15	91*	34717	..	37	....	13.5	13.5	0.792	0.216	13.7	51	
3/13/15	92	33757	80	59	73.8	13.5	29.7	1.261	0.224	12	23	
3/17/15	...	.....	74	65	87.8	11.5	17.5	1.391	0.392	5.7	..	
11/29/15	...	.....	65	53	81.5	13	24.8	1.135	0.228	9.5	22	
11/23/15	...	35042	97	61	63	10.1	13.3	1.309	0.358	5.0	17	Chronic diffuse nephritis; secondary contracted kidney
11/23/15	...	.....	70	41	58.5	8.5	9	0.877	0.292	7.5	..	
11/29/15	92	.....	38	24	63.2	2.9	3.6	0.515	0.272	8.7	23	
12/ 8/15	...	.....	22	15	68.2	2.3	2.1	0.322	0.224	12.8	18	
12/13/15	...	.....	32	21	65.6	4	3.1	0.45	0.254	9.9	22	
12/20/15	...	.....	54	39	72.2	6.3	8.5	0.835	0.289	7.7	..	
4/14/15	96	34637	..	35	....	13.1	9.7	0.749	0.24	11	10	
10/11/15	...	.....	..	41	....	7.3	6.3	0.891	0.341	5.5	3.5	
5/16/16	...	.....	87	49	56.3	....	....	1.049	0.414	3.7	T	



6/ 8/14	171†	21252	..	64	....	....	....	32.2	1.37	0.241	11	..	Primary contracted kidney; arterio-sclerosis
6/19/14	..	.....	..	43	....	....	....	10.5	0.924	0.285	8	..	
9/ 4/15	97*	34631	..	28	....	....	12.9	5.9	0.606	0.245	10.7	18	Primary contracted kidney; uremia
9/20/15	..	.....	..	53	....	....	19.7	24.4	1.141	0.232	12	17	
1/15/16	98	35282	..	51	....	....	14	16.8	1.09	0.266	9	16	Chronic diffuse nephritis; secondary contracted kidney; uremia
1/28/16	..	.....	..	32	....	....	11.7	6.7	0.685	0.265	9	15	
8/26/15	102*	34507	..	35	....	....	5	6.2	0.75	0.302	7	22	Chronic diffuse nephritis; secondary contracted kidney; uremia
9/15/15	..	.....	..	47	....	....	4.5	4.9	1.018	0.457	3	9	
9/27/15	..	.....	..	98	....	....	4.8	5.6	2.102	0.381	0.8	0	
10/14/15	..	34637	110	80	73	....	5.1	7	1.715	0.85	1.5	..	
10/24/15	..	.....	152	78	51.2	....	4.8	8.3	1.67	0.38	1.9	0	
2/10/16	105	.....	..	44	....	....	11.7	7.3	0.941	0.349	5.2	15	Chronic diffuse nephritis; secondary contracted kidney; uremia
2/23/16	..	.....	54	35	64.8	....	8.7	5	0.749	0.336	5.7	7	
3/18/16	..	.....	..	60	....	....	7.4	5.2	1.284	0.563	2	..	
4/ 1/16	..	.....	82	66	....	....	6.1	2.7	1.41	0.36	0.9	..	
1/ 9/14	174*	20575	..	65	....	....	....	12	1.386	0.401	4	0	Chronic diffuse nephritis
4/ 3/14	..	.....	..	211	....	....	....	23.8	4.505	0.324	0.7	0	Chronic diffuse nephritis; uremia
4/28/14	..	.....	..	321	....	....	....	16.7	6.86	1.68	0.2	0	Chronic diffuse nephritis
3/ 4/15	107*	33769	81	64	79	....	7.7	10	1.37	0.434	3.4	13	Primary contracted kidney; uremia
3/12/15	..	.....	96	60	62.5	....	5	5.2	1.284	0.567	1.9	..	
3/26/15	..	.....	102	85	83.3	....	7.5	7.7	1.82	0.665	1.4	2.5	
4/ 9/15	..	.....	94	84	80.4	....	6.2	6	1.796	0.742	1.1	0	
4/20/15	..	.....	192	155	80.8	....	8	2.8	3.318	1.99	0.2	0	
2/ 9/15	108*	33643	40	23	57.5	....	3.3	1.3	0.492	0.436	3.4	28	Primary contracted kidney; uremia

\* Cases ending fatally.

† Cases in which original hypobromite methods were used for analysis.

TABLE 9.—CHRONIC NEPHRITIS—(Continued)

Date	Case No.	Medical No.	Total Non-protein Nitrogen, Mg. per 100 C.c. Blood	Urea Nitrogen, Mg. per 100 C.c. Blood	Percent- age of Total Non- protein Nitrogen Present as Urea Nitrogen	Gm. Urea per Liter of Urine, C	Corrected Rate of Output per 24 Hr. $70 \frac{\sqrt{C}}{P} \times \frac{D}{X}$	Gm. Urea per Liter of Blood, Ur	Coeffi- cient	Index	'Phthal- ein Output, 2 Hr.	Diagnosis
2/25/15	...	.....	78	66	84.9	8.6	4.7	1.415	0.65	1.6	0	Polycystic kidney
9/ 8/15	110*	34684	..	53	....	5	5.7	1.135	0.475	2.8	T	
9/15/15	...	.....	..	40	....	4.8	3.5	0.86	0.46	8	5	
2/ 5/16	111	.....	88	70	81.5	6.6	10	1.498	0.48	2.8	0	Polycystic kidney
2/10/16	...	.....	112	90	80.3	7.4	8.4	1.926	0.665	1.4	0	
2/19/16	...	.....	86	69	80.3	5.5	4.3	1.476	0.714	1.3	0	
3/ 3/16	...	.....	92	62	67.4	6.8	13.6	1.327	0.359	5	0	Chronic diffuse nephritis; secondary contracted kidney; uremia
3/11/16	...	.....	..	52	....	5.7	5.7	1.112	0.466	2.9	0	
3/12/16	...	.....	..	57	....	5	2.4	1.22	0.793	1	0	
9/14/15	112*	34725	..	76	....	6.1	7.5	1.629	0.597	1.8	T	Chronic diffuse nephritis; secondary contracted kidney; uremia
9/24/15	...	.....	..	120	....	5.8	9.9	2.568	0.815	0.9	0	
10/ 4/15	...	.....	181	163	90	....	....	....	....	..	..	
1/20/16	114*	35306	..	142	....	8.1	24.5	3.143	0.689	1.5	0	Chronic diffuse nephritis; secondary contracted kidney; uremia
1/26/16	...	.....	..	178	....	9.6	9.7	3.818	1.23	0.4	0	
3/ 8/16	116*	35566	..	89	....	6.9	6.6	1.917	0.742	1.2	T	
3/18/16	...	.....	..	118	....	9.4	8.7	2.523	1.31	0.4	T	Chronic diffuse nephritis; secondary contracted kidney
8/19/15	117*	.....	..	53	....	5.4	2.3	1.131	0.75	1.1	..	



8/28/15	...	....	..	58	....	7.2	17	1.88	0.458	3	18
9/ 6/15	...	....	..	63	....	6	9.6	1.349	0.425	3.5	14
10/ 7/15	...	....	125	99	79.2	4.4	3.5	2.119	1.125	0.5	5
10/14/15	...	....	145	126	87	6.4	4.3	2.7	1.305	0.4	..
10/21/15	...	....	123	103	83.9	8.3	6.9	2.2	0.836	0.9	7
10/28/15	...	....	66	49	74.3	3.4	4	1.049	0.524	2.3	5
11/ 5/15	...	....	55	32	58.2	2.1	1.7	0.685	0.525	2.3	5
11/ 9/15	...	....	88	64	72.8	2.4	2.9	1.37	0.8	1	..
11/16/15	...	....	95	63	66.4	5.1	4.6	1.349	0.631	1.6	..
11/23/15	...	....	79	55	69.6	2.9	3.3	1.177	0.65	1.5	6
11/30/15	...	....	41	30	73.1	1.9	2.1	0.642	0.445	3.2	..
12/ 7/15	...	....	82	23	71.9	1	0.9	0.492	0.52	2.4	..
12/11/15	...	....	86	26	72.3	1.7	1.7	0.557	0.43	3.5	..
12/30/15	...	....	..	32	....	2.3	2.9	0.676	0.396	4.1	..
1/13/16	...	....	68	52	76.5	5	2.7	1.112	0.682	1.4	5
1/25/16	...	....	63	45	71.5	3.9	3.6	0.964	0.509	2.5	..
2/12/16	...	....	..	79	....	4.1	0.8	1.69	1.872	0.2	..
2/24/16	...	....	192	149	77.6	9.8	5.4	3.187	1.37	0.3	T
3/ 3/16	...	....	216	170	....	10.8	5.2	3.64	1.591	0.2	T
3/10/16	...	....	..	174	....	6.1	1.9	3.725	2.67	0.1	..
3/25/14	177††	20903	..	173	....	10.6	13.9	3.725	0.935	0.6	0
4/16/14	...	....	..	419	....	15.9	1.3	8.96	7.95	..	0

Primary contracted kidney; uremia

\* Cases ending fatally.  
† Cases in which original hypobromite methods were used for analysis.

In vascular nephritis death is due so frequently to extrarenal accidents (vascular or cardiac) that extreme renal insufficiency is somewhat unusual. In such cases the presence of a relatively unimpaired renal function should not be regarded as necessarily warranting a favorable prognosis. For example, Case 71 was a cerebral arteriosclerotic, with some evidences of a vascular nephritis. The functional tests showed slightly damaged kidney (55 per cent. phenolsulphone-phthalein excretion, coefficient 0.141), yet he died two weeks later of cerebral hemorrhage. We have had very little opportunity of following the cases of primary contracted kidney over long periods. Such patients have usually come for consultation on account of symptoms referable to hypertension rather than to any renal insufficiency and have not remained in the hospital. Of fifty-eight patients examined, forty-eight had coefficients below 0.2. Thirty-three of these have been traced, and of these; only five are dead. One died of uremia, three of cerebral hemorrhage, and one with extreme myocardial insufficiency. Of the ten patients with coefficients above 0.2, nine have been followed and eight of these are dead. Six died in the hospital in coma, two died of cerebral hemorrhage shortly after their discharge. Extreme myocardial insufficiency was present in three of the eight patients during their terminal illness. It is evident from the foregoing that functional signs of renal insufficiency occur only in the last stages of the disease. Coefficients above 0.175 in chronic vascular nephritis justify a very guarded prognosis.

The cases of chronic diffuse nephritis can be divided into three distinct types:

1. Those which remain stationary over prolonged periods, in spite of an outspoken renal insufficiency. The functional tests have a peculiar value in identifying these cases and in giving evidence of the onset of a progressive lesion, which under ordinary circumstances is the final event.

2. Other cases show a progressive course, with constantly increasing renal insufficiency. In these the coefficient and phenolsulphone-phthalein test enable us to follow the rate of progress and thus assist in an accurate prognosis.

Seventy-one determinations have been done on thirty-nine such cases in which the coefficient was below 0.2. Thirty of these patients have been traced and ten have died, six in uremic coma. Seventy-six determinations have been done on twenty-six patients with coefficients above 0.2. Twenty-one of these patients have been traced and fifteen of them are dead. Eleven died in uremia within a short time of observation, and most of them had more or less severe myocardial insufficiency. It is evident from the foregoing that here again individuals with a coefficient above 0.2 have an expectation of life measured in

months, although the danger is not so immediate as in those cases of vascular nephritis with an equally high coefficient.

3. In three cases (Cases 23, 26 and 35) the functional studies gave no inkling of the severity of the lesion. These were all examples of rapidly progressive uremia. The history was the same in every instance. There were severe headaches, visual disturbances, nausea, vomiting and loss of weight. Hypertension, cardiac hypertrophy, diffuse vascular thickening, and extreme grades of albuminuric retinitis were present in every case. They showed normal blood ureas, coefficients below 0.14 and phenolsulphonephthalein excretions above 40 per cent. These patients all died in convulsions within five months of the time of observation. None of these cases came to necropsy. From the history, Case 114 was probably another member of the group, seen, however, in the last stages. At necropsy a moderate grade of secondary contracted kidney was found. In these cases a comparison of the severity of the symptoms and physical signs, with the mild grade of functional impairment, gave a perfectly definite picture. The absence of outspoken functional impairment should not be misleading. The prognosis in these cases was measured in weeks.

While in a majority of cases the functional findings are of great assistance in the making of an immediate prognosis (as in those cases with a coefficient above 0.2), we are still at a great disadvantage in those cases having only a moderate grade of impairment. Under ordinary conditions a low coefficient (below 0.12) which remains at approximately the same level for some time justifies a good prognosis. One should be extremely careful in giving a favorable opinion with values above 0.15, as a coefficient at this level means that a large proportion of the active renal tissue has been damaged, and any extra strain put on the organ will undoubtedly cause serious renal insufficiency.

Practically every patient with a coefficient steadily above 0.35 has died within six months. Every one of these patients, however, had such outspoken signs of renal insufficiency that the information gained by functional study was merely of a confirmatory nature.

#### SUMMARY

1. The laws of function are not followed with mathematical exactness in young and active individuals, but under routine conditions they are remarkably accurate. They are correct in principle.

2. The coefficient of urea excretion is subject to certain variations in normals, but any value below 0.06 or above 0.09 should be regarded as abnormal unless the excessive variation can be readily explained.

3. The coefficient is absolutely independent of the blood urea concentration. Its level is governed by the condition of renal function.



4. The coefficient is depressed in fever, in hyperthyroidism, in hypertension with early changes in the renal arterioles, and in early chronic diffuse nephritis. The depression is an evidence of increased renal activity due to irritation.

5. The coefficient is raised in myxedema.

6. There is an increase of the coefficient in myocardial insufficiency. Opinions are divided as to whether this is the effect of an extrarenal factor (the circulation) or whether there is a definite anatomic lesion in the passively congested kidney.

7. The coefficient is above normal in nephritis with renal insufficiency. This increase is more evident in chronic diffuse nephritis than in the vascular type, due to the greater frequency of renal insufficiency in the former cases. The coefficient shows an increase long before there is any evidence of nitrogen retention in the blood. The coefficient gives an excellent means of following the changes in renal function and of measuring the rate of progress of the disease.

8. There is a marked uniformity in the results of the phenolsulphonephthalein test and the coefficient in all stages of nephritis. In the later stages there is also a close agreement between the nonprotein nitrogen of the blood and the coefficient.

9. In a few severe cases the coefficient varies without there being any evident change in the clinical condition; the causes of these variations have been discussed.

10. The prognostic value of the coefficient is considerable. Values above 0.2 are seen only in the severe cases, while constants persistently above 0.3 are found only in persons with a maximal impairment of renal function. A coefficient above 0.2 has a graver import in vascular nephritis than in that of chronic diffuse type.

11. For an accurate prognosis repeated determinations of the coefficient are of the greatest importance.

## THE ACTION OF THE SEVERAL "FEMALE REMEDIES" ON STRIPS OF THE EXCISED HUMAN UTERUS\*

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In a recent paper<sup>1</sup> were presented the actions on the excised uterus of the guinea-pig of a long list of drugs that have been reputed to possess certain rather indefinite actions on the uterus. Since then there has been an opportunity to investigate the actions of a few of the same drugs on strips of the human uterus in the same manner. The number of experiments in this series is rather limited, but as the results agree qualitatively with those on the guinea-pig uterus and as a large percentage of the strips from the human uterus do not contract at all, the number was thought to be sufficient to warrant conclusions for this work. The experiments were made on strips from four nonpregnant uteri and from one fallopian tube. The uteri were obtained from the surgical services of the Omaha hospitals<sup>2</sup> and were used on the same day; one specimen was used on the second day also. Four specimens were from patients past the menopause and the fifth from a patient 28 years of age; the results were similar in each case.

Character of the Movements of Strips from the Human Uterus: Gunn<sup>3</sup> has previously noted that strips from the human uterus contract similarly to those from the guinea-pig; his tracings, however, agree with mine that the movements are of much less magnitude as a rule, occasionally being no more than wavy lines. Very frequently strips showed no contractions at all. Possibly the pregnant human uterus would give a larger percentage of active strips, as is the case with the guinea-pig uterus.

The following drugs were examined: *Pulsatilla pratensis* (pulsatilla), *Aletris farinosa* (unicorn root), *Caulophyllum thalictroides* (blue cohosh), *Cnicus benedictus* (blessed thistle), *Viburnum prunifolium* (black haw) and oil of valerian. It was found that pulsatilla, aletris and oil of valerian (Fig. 1) lowered the amplitude of the excursions or completely inhibited them; but the action was not so prompt or so great as on the guinea-pig uterus in the same concentration. Caulo-

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1. Pilcher, Burman and Delzell: THE ARCHIVES INT. MED., 1916, **18**, 557.

2. From the services of Drs. Findley, Jonas, Waters and Mosher.

3. Gunn, J. A.: Proceedings of the Royal Society, 1914, **87**, 551.

phyllum (Fig. 2) put the strips into tonic contraction, but again the action was less than on the guinea-pig; the tone was not increased above the maximum, as was frequently the case with the guinea-pig. *Cnicus benedictus* and *viburnum* were inactive. The latter was tried in but a single experiment, however.

The Experimental Data: With the *pulsatilla* there was a gradual lowering of the excursions with the 1 to 1,000 solution in one case, and from the 1 to 500 and the 1 to 250 solution in a second. A third strip was practically unaffected by the 1 to 1,000 solution.

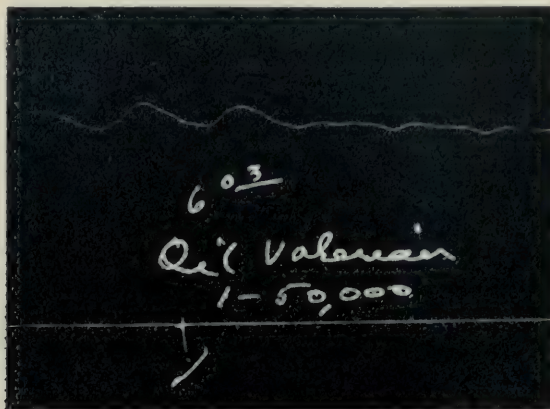


Fig. 1.—Oil of valerian on a strip of human uterus; at 1 it was applied to make a 1 to 50,000 solution.

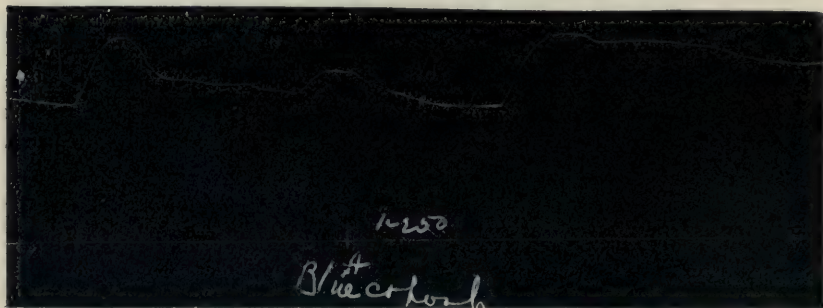


Fig. 2.—*Caulophyllum thalictroides* (blue cohosh) on a strip of human uterus; applied at A to make a 1 to 250 solution.

With *aletris* a single strip gradually ceased to contract when placed in a 1 to 500 and 1 to 250 solution.

With oil of valerian the contractions of five strips promptly ceased when placed in a 1 to 25,000 solution, and the inhibition was permanent; there was considerable depression from a 1 to 100,000, and a 1 to 50,000 concentration in one case. Stronger solutions were correspondingly more active. Three experiments were on strips from the fallopian tube, which were also depressed. These were the only experiments made on the tube. The larger number of



experiments were made with the oil of valerian as it was used to insure the accuracy of the method in connection with other work.

The typical action of *caulophyllum* was exhibited in four experiments with a 1 to 500 concentration and in a single case with a 1 to 250 solution of the evaporated fluidextract.

*Cnicus benedictus* was inactive in three cases with the 1 to 500 solution and in a single case with the 1 to 100 solution of the evaporated fluidextract.

*Viburnum prunifolium* was inactive in but a single experiment with a 1 to 500 concentration.

#### COMMENT

The results show that the drugs examined act on strips from the human uterus in the same direction as on the guinea-pig uterus; but that more concentrated solutions are required to produce the same effect. They thus strengthen the argument of the previous paper that it is highly improbable that these drugs could exhibit a similar action on the intact uterus in doses that could be tolerated by the patient. But a limited number of drugs of this group, the so-called uterine tonics and sedatives, have been examined. But as the results agree qualitatively with those of the previous work on the guinea-pig, it seems safe to conclude that the others would have the same action on strips of the human uterus as from the guinea-pig uterus, but probably to a lesser degree.

#### CONCLUSIONS

The drugs examined act on strips of the isolated human uterus in the same direction as on the guinea-pig uterus, but to a much less degree. *Aletris farinosa*, *pulsatilla pratensis* and oil of valerian depress the activity of the strips; *Caulophyllum thalictroides* throws the strips into tonic contraction; *Cnicus benedictus* and *Viburnum prunifolium* are inactive.

# THE PRESENT SIGNIFICANCE OF THE AMINO-ACIDS IN PHYSIOLOGY AND PATHOLOGY\*

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## CHEMICAL NATURE OF THE AMINO-ACIDS

This discussion is inserted because it will be necessary, for the ready understanding of the later parts, that speaker and audience shall have in mind from the same point of view two or three significant chemical characteristics of the amino-acids as a class.

We know, chiefly as the result of the researches of Fischer,<sup>1</sup> Kossel, and their collaborators, that the amino-acids are the units or building stones out of which the protein molecule is constructed. They are the final products obtained when proteins are hydrolyzed by strong acids, or by the action of pepsin, trypsin and erepsin in the alimentary canal. In the characteristic points of their structure the amino-acids are all alike. That is, they belong to a type, and we have only to understand the type in order to become fairly well acquainted with them all. We have pictured in Figure 1 what may be designated as a decapitated amino-acid. It is the portion of the molecule which is common to all the amino-acids, and its formula expresses the chemical properties which are characteristic of them as a class. Of these properties, the most striking are due to the occurrence in the same molecule of an amino group, with a basicity like that of ammonia, and an acid group with an acidity like that of acetic acid. Hence from these two groups the name, "amino-acid." The amino and acid groups are joined by a single carbon atom, which serves as a bridge between them. This structure occurs in every amino-acid. The central carbon atom is the center of the entire molecule. It is flanked on one side by the amino group, on the other by the acid group, a third valence is occupied by an insignificant hydrogen atom, while to the fourth, which in the decapitated formula is left pointing upward and unattached, is fastened what we may term the head of the molecule. This is different in every amino-acid. It is the source of the individuality of each. There are eighteen varieties of such heads, as may be seen by glancing at Figure 3, and, corresponding to them, eighteen distinct amino-acids, each possessing the common group characteristics indicated by the

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1. Fischer, Emil: Untersuchungen über Aminosäuren, Polypeptide, und Proteine, Ber. d. deutsch. chem. Gesellsch., 1906, **39**, 530.

body, and in addition, another set of chemical characters entirely belonging to itself and indicated by the structure of the head.

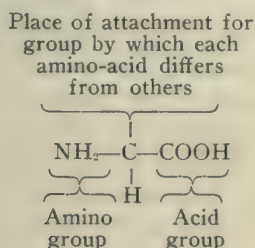


Fig. 1.—Formula of a decapitated amino-acid.

Our interest centers mainly, however, on the family body and its characteristics. Of these we have already mentioned the basic and acid properties combined in the single substance. I would call your attention to one other, also dependent on the simultaneous presence of amino and carboxyl acid groups, and this is the ability of the amino-acids to dovetail themselves together and form molecular chains of infinite length. It is this ability which makes possible the existence of such complex substances as proteins and protoplasm. We have indicated in Figure 2 the mechanism by which the linking of the units in the chain is accomplished. It represents the coupling of two of the amino-acids, alanin and glycoll. We see that the amino group of the alanin condenses with the acid group of the glycoll, with elimination of a molecule of water. The result is that the two amino-acids are linked together and form a peptid, alanyl-glycoll, called a dipeptid because it contains two amino-acids. This peptid, however, like the original amino-acid, still contains one free amino group at one end and an acid group at the other. It can therefore couple on another amino-acid at either end. These could still condense with two more, and so on ad infinitum. Protein molecules are chains composed of scores or hundreds of amino-acids joined together in this way.

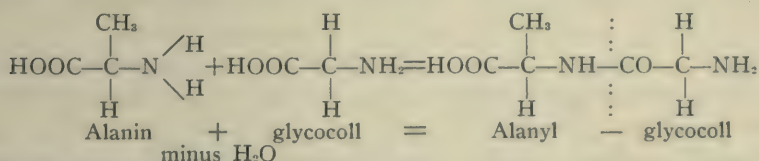


Fig. 2.—Coupling of the amino-acids, alanin and glycoll, to form the dipeptid, alanyl-glycoll.

When proteins are hydrolyzed, or digested, by trypsin, for example, the links of the chain are broken apart and we have, first, somewhat shorter chains, the albumoses; then still shorter chains, the peptones, which are mixtures of peptids, and finally the separate amino-



acids. This successive breaking down of the long chain into shorter chains and finally into the separate links constitutes the process of digestion. The building up of new protein consists of the reverse, namely, the linking together of the amino-acids into new chains.

To show at the same time the nature of the protein structure and of the different amino-acids which take part in it, I have placed in Figure 3 the structural formula of an imaginary protein containing one molecule of each of the eighteen known amino-acids. We see along the bottom of the row the repetition of the familiar family body, the central carbon atom flanked in each case by the accompanying amino and acid groups. Above, however, we have the individual heads of the different units in great variety. We might liken the protein chain to a long train of automobiles, all with small, black, uniform bodies, but with tops of eighteen different shapes, and of three different colors, according to whether the properties they carry are acid, basic, or neutral. It will be noted that among the amino-acids the neutral party is in the great majority, which fact accounts in part for the approximately neutral reaction of most proteins.

Whenever a peptid linking in the protein chain is broken by hydrolysis, we have at once one amino group and one acid group set free. Chemically stated, the hydrolysis or digestion of a protein consists in the splitting of some or all of the peptid linkings between its amino-acids, with the formation of new acid and amino groups in exact proportion to the extent of the digestion. In order to determine the occurrence and extent of digestion with exactness, therefore, we must determine either the amino groups or the acid carboxyl groups that are formed by the process. Only by such means can we obtain results capable of exact chemical interpretation. The various physical methods of colloid coagulation, viscosity determinations, precipitation, etc., useful though they have been, are only rough and indirect measures of the chemical process which constitutes digestion. For a direct measure we must determine either the amino or the acid groups which are set free. Furthermore, our only chemical means for estimating the complexity of any intermediate product, such as a peptone or albumose, lie in determining the ratio between the free amino or acid groups which it possesses and those which are found after it has been completely hydrolyzed. Thus, the amino nitrogen of a dipeptid, composed of two amino-acids, is doubled by hydrolysis, that of a tripeptid is tripled, of a tetrapeptid quadrupled, etc.

All of the above facts concerning the relationship between the progress of digestion and the uncovering of amino and carboxyl groups were recognized over ten years ago, as soon as Emil Fischer<sup>1</sup> had demonstrated the peptid nature of the protein molecule. As the result of this knowledge, the desirability for quantitative methods for the



determination of either amino or carboxyl groups became evident. As generally occurs, when the need became clear, the methods were invented. As the use of these methods is most intimately connected with the experimental work of which I shall speak, I shall stop here for a moment to discuss the two which have found most general application.

The first was published by Soerensen in 1908.<sup>2</sup> It was based on the fact, discovered by Schiff,<sup>3</sup> that formaldehyde added to the water solution of an amino-acid combines with the amino group, and that in consequence the amino group loses its alkalinity. As the formaldehyd itself is neutral, the effect of the reaction is to reduce the amount of titratable alkali, or increase the titratable acid by an amount equivalent to the amino nitrogen present. Soerensen tested this method with practically all the known amino-acids, and worked out the details necessary for attainment of the most accurate results. In brief, the formaldehyd titration of Soerensen is performed by rendering the solution of amino-acid neutral to litmus, adding formaldehyde, and then titrating against phenolphthalein the acid which has been set free by the removal of the alkaline capacity of the amino groups. The ingenuity and simplicity of this method led to its immediate adoption by biologic chemists, and many investigations of value have already been conducted with it.

The second method was published by myself in 1909.<sup>4</sup> It rested on the well-known reaction of amins with nitrous acid, as the result of which the nitrogen of the amino group is transformed into nitrogen gas. In order to determine the amount of amino nitrogen present, therefore, one has merely to add nitrous acid and measure the volume of nitrogen gas which is set free by the reaction. The principle is similar to that of urea determination by the hypobromite method. We were able so to fix conditions that the reaction is complete in three minutes. A considerable amount of nitric oxid gas is evolved by spontaneous decomposition of the nitrous acid, and this gas is used to drive the air out of the apparatus before the amino-acid solution is admitted. At the end of the reaction the nitric oxid is absorbed by permanganate solution, and the pure nitrogen gas given off by the amino group is measured.

In the apparatus which finally proved the most convenient the entire process can be carried out in a few minutes and results obtained

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2. Soerensen, S. P. L.: *Enzyme Studien*, *Biochem. Ztschr.*, 1908, **7**, 44.

3. Schiff, Hugo. *Ann. d. Chem.*, 1900, **348**, 59.

4. The principle of the method and the first form of the apparatus were described in the *Proc. Soc. Exper. Biol. and Med.*, Dec. 15, 1909. Details, improvements, and applications to micro-analysis have been described in the *Jour. Biol. Chem.*, 1911, **9**, 85; *ibid.*, 1912, **12**, 275; *ibid.*, 1913, **16**, 121; *ibid.*, 1915, **23**, 407.



with a high degree of accuracy. As compared with the formaldehyd titration, the nitrous acid method has the disadvantage that it requires a special apparatus. It has several advantages, however, in that the readings can be made with a higher degree of accuracy, that the determination is not interfered with by the presence of colored substances in the solution, and that accurate results can be obtained with extremely small amounts of material. With a micro-apparatus readings significant to 0.001 mg. of amino nitrogen can be obtained, while 0.25 mg. is as small an amount as can be determined by the formaldehyd method. Because of these advantages, which were important in the conditions under which we worked, we have used the nitrous acid method in our own investigations on the fate of protein digestion products in the body.

The table gives an idea of the nature of the results obtained in following the course of a protein digestion. It will be noted that there is a slight amount of amino nitrogen present before any digestion has occurred. This is due to the fact that one of the two amino groups of the lysin is free in the protein molecule. This was demonstrated in our laboratory by Birchard,<sup>5</sup> who showed that in a representative series of proteins an amount of free amino nitrogen equal in all cases to half the lysin nitrogen could be demonstrated by the nitrous acid method. It will be noted in Figure 3 that one amino group in the guanidin nucleus of arginin is also free. This guanidin  $\text{NH}_2$ , for an unexplainable reason, however, fails to give some of the characteristic reactions of amino groups in general. It does not react with nitrous acid, or with formaldehyd in the Sorensen titration, and therefore is responsible for none of the free amino nitrogen that is noted in the protein even before digestion has begun.

INCREASE OF AMINO NITROGEN DURING TRYPTIC DIGESTION OF  
4 PER CENT. EDESTIN SOLUTION

Hours	C.c. of N Gas from 10 C.c. Solution	Per Cent. of Hydrolysis
0	1.20	0
2	7.62	14.8
4	8.92	18.2
20	12.62	27.4
80	19.56	47.3
Complete hydrolysis with HCl	40.25	100

The foregoing finishes our discussion of the organic chemistry of the amino-acids and the methods used for their determination. We shall now turn our attention to a study of the fate of protein digestion products in the body. This study has been guided by the conception of the relationship between proteins and amino-acids, which I

5. Van Slyke and Birchard: Jour. Biol. Chem., 1914, **16**, 539.

have just outlined, and was carried out to a large extent with the aid of the nitrous acid method for the experimental investigation of that relationship.

#### PHYSIOLOGY OF THE AMINO-ACIDS

At the time these investigations were begun the old Liebig theory of protein metabolism had already long been abandoned, and in place of it there was considerable confusion. Liebig's belief was very simple. He thought that the food proteins were incorporated directly into the tissues of the animal. The only necessary preparation was that of putting the proteins into solution in order that they might be absorbed, and this purely physical change was the sole object of digestion. The better understanding of gastric digestion, Kühne's<sup>6</sup> discovery of trypsin, and finally Cohnheim's<sup>7</sup> demonstration of the action of erepsin in reducing proteoses to amino-acids, led inevitably to the conclusion that food proteins undergo not only physical but also chemical change in the alimentary canal, namely, that digestion is a hydrolysis, and that the hydrolysis proceeds partially, if not entirely, to the stage of amino-acids before the products are absorbed.

The results of a century of laborious research by many keen investigators from Spallanzani and Beaumont to Cohnheim may be summarized as follows: The proteins enter the stomach and are digested to the stage of albumoses; that is, the long protein chain of amino-acids is broken into somewhat shorter, but still very long chains, and thereby the protein, which is usually insoluble, is transformed into soluble albumoses. The latter are not absorbed, however. London,<sup>8</sup> working in St. Petersburg, has shown conclusively that no absorption takes place from the stomach during normal digestion. The albumoses all pass down into the intestine, where they meet the pancreatic juice and are split, partly into short chains of two or three amino-acids each, and partly entirely to free amino-acids. That free amino-acids constitute a considerable part of the products of intestinal digestion was demonstrated by Abderhalden,<sup>9</sup> who isolated most of the known amino-acids from intestinal contents. That the entire mass of products, aside from the free amino-acids, consists of short chain peptids was shown by White and myself<sup>10</sup> with the nitrous acid method in the case of one of the lower animals, the dogfish. This work was done in 1910 at Woods Hole. Shortly after, London, by means of the formaldehyd titration, obtained results of the same nature with dogs.<sup>11</sup> Finally,

6. Kühne: *Virchows Arch. f. path. Anat.*, 1867, **39**, 130.

7. Cohnheim: *Ztschr. f. physiol. Chem.*, 1901, **33**, 451.

8. London: *Ztschr. f. physiol. Chem.*, 1913, **87**, 313.

9. Abderhalden: *Ztschr. f. physiol. Chem.*, 1912, **78**, 382.

10. Van Slyke and White: *Jour. Biol. Chem.*, 1911, **9**, 209.

11. London and Rabinowitsch: *Ztschr. f. physiol. Chem.*, 1912, **74**, 305.

either before or after entering the intestinal wall, the products encounter a third hydrolytic enzyme, erepsin, which is capable of carrying the hydrolysis to the stage of amino-acids still nearer completion.

You will note that the above summary, which indicates the stage of our knowledge five years ago concerning the mechanism of protein nutrition, stops short against the intestinal wall. This was, as a matter of fact, the place where facts ceased and theories began. What happened to the amino-acids and peptids after they were absorbed from the intestine was not known. Neither amino-acids nor peptids could be detected in the blood. As the veteran Pflüger pointed out, the failure to detect either amino-acids or peptids in the circulation might well be due to a lack of sufficiently delicate methods, for the flow of portal blood is so fast that even a maximum absorption of nitrogen might cause but a very small concentration in the blood at any given moment. Folin,<sup>12</sup> in his classic paper on the theory of protein metabolism, published eleven years ago, took the same stand. In order to fill the gap in experimental results, however, other authors proposed two theories: (1) The amino-acids are decomposed into ammonia and nonnitrogenous residues while passing the intestinal wall; (2) they are synthesized into blood protein. The latter theory, it will be noted, was particularly convenient, because it not only explained the failure to find amino-acids in the blood, but also gave the source of the blood proteins. It was especially championed by Abderhalden.

The development of adequate methods, however, showed that Pflüger and Folin were right, and both of the above explanatory theories became unnecessary. The first theory received its death blow at the hands of Folin.<sup>13</sup> With the extremely delicate colorimetric method for the determination of ammonia which he devised, he was able to show that absorption of amino-acids from the intestine is accompanied by no increase whatever in the ammonia of the portal blood. When put to the rigid test of quantitative experiment, the deaminizing ability of the intestine vanished into thin air.

The fate of the resynthesis theory was similar. The sole foundation on which it rested was the negative results of attempts to find in the blood digestion products, either peptones or amino-acids. As soon as quantitative methods, namely, the formaldehyd titration and the nitrous acid method, were applied, however, it was shown by investigators working independently with each that the blood does contain amino-acids and that they increase markedly during digestion. This was shown by Delaunay,<sup>14</sup> working in Bordeaux, with the for-

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12. Folin: *Am. Jour. Physiol.*, 1905, **13**, 117.

13. Folin and Denis: *Jour. Biol. Chem.*, 1912, **9**, 246.

14. Delaunay: *Thèse de Bordeaux*, 1910; *Abstr. Arch. d. mal. de l'ap. dig. et de la nutr.*, 1911, **5**, 218.



maldehyd method, and by Meyer and myself<sup>15</sup> in the Rockefeller Institute with the nitrous acid method. The normal amino-acid concentration in the blood of both dogs and men is about that of sugar, about 0.1 per cent., and it may in dogs be nearly doubled in the portal blood as a result of a heavy protein meal.

The force of these results was further strengthened by Abel, Rowntree, and Turner<sup>16</sup> in their remarkable experiments with vivi-diffusion. These experimenters passed the blood of living dogs through collodion tubes immersed in salt solution, into which the non-colloid substances of the blood diffused. From the diffusing substances thus obtained they were able to separate in pure condition and identify several of the individual amino-acids. Abderhalden then also applied dialysis to blood and was able to obtain most of the amino-acids in sufficient amounts to identify them.

After entering the circulation, amino-acids disappear from it again very quickly. Within five minutes after 12 gm. of alanin had been injected into the vein of a dog, 90 per cent. had disappeared from the circulation. A similarly rapid removal must occur during digestion, otherwise amino-acids would accumulate in much larger amounts in the blood than we observe. The question then naturally raised itself as to what becomes of the amino-acids when they vanish from the circulation. Are they decomposed in the blood; are they at once somewhere synthesized into new protein; are they chemically incorporated into the complex molecules of the tissue protein; or are they merely absorbed by the tissues in general or by certain tissues in particular without undergoing any immediate change?

Analysis of the tissues of dogs which had received intravenous injections of known amounts of amino-acids answered these questions in favor of physical absorption.<sup>17</sup> In one experiment, which will serve as an example, the amount of amino nitrogen injected in the form of hydrolyzed casein was sufficient, if distributed evenly throughout the body, to raise the average amino nitrogen content of the tissues 40 mg. per 100 gm. of tissue. The increases actually noted were muscles, 27 mg.; liver, 60 mg.; kidney, 60 mg.; intestinal wall, 50 mg. That the absorbed amino-acids could not have been in even loose chemical combination in the tissues was shown by the fact that they could be extracted by such mild agents as water, hot or cold, or dilute alcohol. They must have been held merely by physical forces.

The tissues, despite the great rapidity with which they absorbed amino-acids from the blood, never removed them from it completely.

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15. Van Slyke and Meyer: *Jour. Biol. Chem.*, 1912, **12**, 399.

16. Abel, Rowntree, and Turner: *Jour. Pharmacol. Exper. Therap.*, 1914, **5**, 275.

17. Van Slyke and Meyer: *Jour. Biol. Chem.*, 1913, **16**, 197.

An equilibrium is reached when, stated very roughly, the tissues contain about ten times the percentage of amino-acid present in the blood. From the fact that they are so much more concentrated in the tissues than in blood it is evident that the process by which they are picked out of the circulation is not a mere diffusion. If it were, we should find approximately equal concentration in both tissues and blood. The physical process by which the exchange between blood and tissues is carried out has not yet been definitely classified with any of the physical or physicochemical phenomena with which we are familiar. Until it is explained by such classification we cover our ignorance of the real nature of the process by giving it the general name, "absorption."

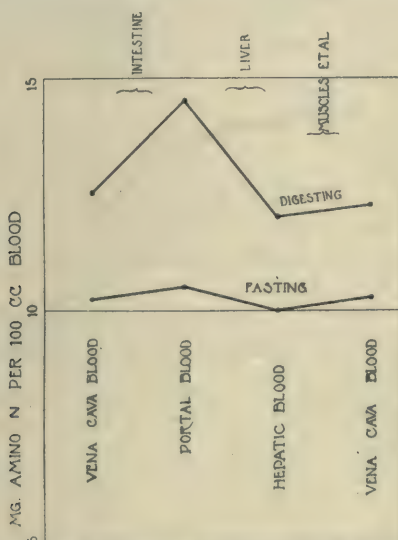


Fig. 4.—The amino-acid content of the blood during fasting and protein digestion; average of results from six fasting and six digesting dogs.

We have now followed the protein digestion products, that is, the amino-acids, from the alimentary tract past the wall of the intestine into the blood stream and from the blood stream into the tissues. But we have yet reached only a temporary stopping place. Most of the protein nitrogen in the daily diet of an adult is excreted within twenty-four hours as urea; and Levene and Kober<sup>18</sup> found that when single amino-acids were fed to dogs they were excreted entirely as urea. It is evident that whatever stopping place the greater part of these products find in the tissues is only a temporary refuge preliminary to their speedy destruction and elimination.

Present knowledge points to the liver as the organ which is most active both in absorbing amino-acids from the blood stream during

18. Levene and Kober: *Am. Jour. Physiol.*, 1908, **23**, 324.

normal digestion and in submitting them to the preliminary chemical alterations which precede elimination as urea or storage as reserve protein. Figure 4 shows that during digestion there is a greater fall in amino nitrogen during the passage of the liver (difference between portal and hepatic blood) than during passage through the entire remainder of the body (difference between arterial and vena cava blood). The liver is the organ to which the portal blood first comes with its newly acquired amino-acids and it is the liver that takes the lion's share of them. It follows as a necessary corollary that the liver must either store immense amounts of them after a heavy protein meal, or must quickly transform them, either into urea for elimination, or into reserve protein for storage.

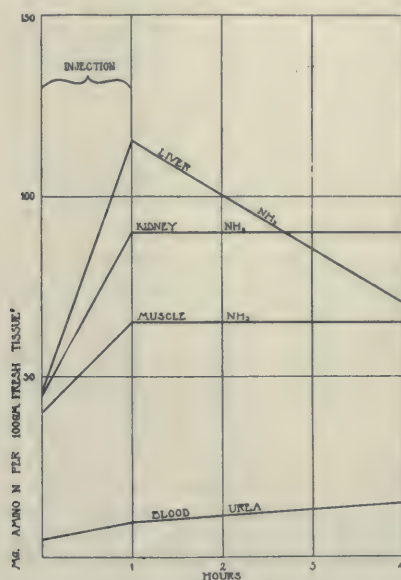


Fig. 5.—The absorption and retention, by different tissues, of amino-acids injected intravenously.

Further experiments have shown that the liver does not store amino-acids as such to an appreciable extent. Chemical transformation follows very quickly after absorption. This was shown in three different ways:

First, the tissues of dogs in fasting condition were compared in respect to their amino-acid content with those of dogs which were digesting or had digested large amounts of protein. It was found that neither the livers nor other tissues of the fed animals contained a definitely greater store of amino-acids than did the tissues of the fasting animals. The digesting dogs must have either destroyed or condensed into protein practically all the amino-acids which they absorbed,



and have done so at a rate which was nearly parallel with that of absorption.

Second (Fig. 5), dogs were injected intravenously with such amounts of amino-acids that the amino nitrogen content of all the tissues was raised considerably. Samples of muscular tissue, a lobe of the liver, and a kidney, were taken immediately after the injection, and again three or four hours later, the animals being kept under ether anesthesia by the Meltzer-Auer insufflation method. It was found that whereas the muscles and kidney still held after four hours all the amino-acids which they had absorbed, those taken up by the liver had disappeared. They had not been excreted, and there was no reason for assuming that they had been transferred to any other organ. The disappearance of the liver amino-acids was accompanied by a rise in

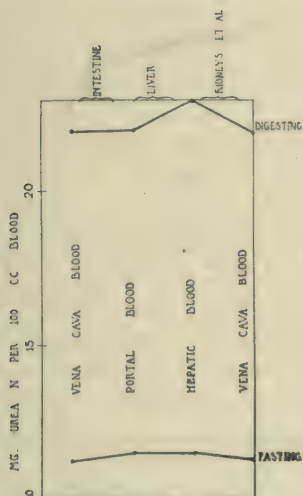


Fig. 6.—The blood urea during fasting and protein digestion; average of results from six fasting and six digesting dogs.

the blood urea. The conclusion seemed justified that the liver can destroy amino-acids at a rate very much greater than the muscles and that at least a portion of the nitrogen of the amino-acids disappearing from the liver is converted into urea.

Third, comparison of Figures 4 and 6 shows that the blood in passing through the liver takes from it about as much nitrogen in the form of urea as it gives to it in the form of amino-acids.

All the above experiments emphasize the activity of the liver in metabolizing amino-acids, from which it produces urea, apparently the most abundant product.

On the other hand, however, it does not appear that urea formation is a process entirely confined to the liver. Folin's collaborators, Fiske

and Summer,<sup>19</sup> have observed an increase in the blood urea when the liver was cut out of the circulation. Nencki and Pavlov<sup>20</sup> in 1893 showed that a dog deprived of its liver could still form and excrete urea, though in decreased amounts. It appears therefore that present experimental results may be interpreted by stating that the most active center of amino-acid transformation, and of urea formation, appears to be the liver, but that the localization of the function is not absolute, and these processes also occur to some extent in other organs.

The next question to be raised is, does the liver, during the digestion of a protein meal, wait till the other tissues are saturated with amino-acids, and then begin to destroy the unnecessary excess, which is not needed by the organism, or does it begin to destroy the first that reach it in the portal blood? Unreasonable as it may seem, the latter

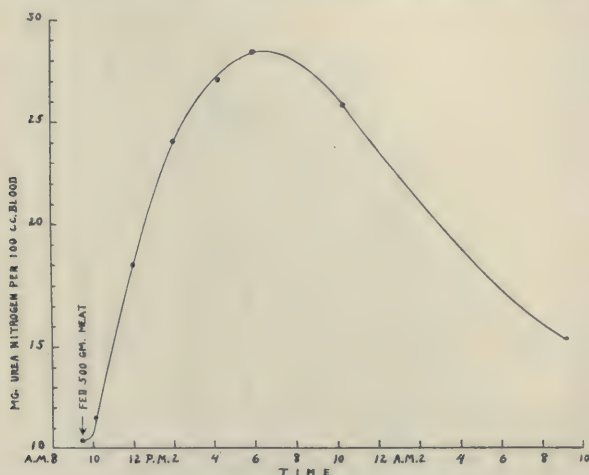


Fig. 7.—Time curve of blood urea changes during protein digestion.

behavior is what we observe. In order to test this point urea determinations were made at short intervals on blood from dogs which, after two days' fast, had received heavy meals of meat. It was found in all cases that the urea began to rise almost immediately after the meat was consumed. There was no interval of waiting commensurate with what might be expected if urea formation were delayed until after the tissues in general had replenished their store of amino-acids. It was furthermore shown by roentgenograms that the blood urea began to rise at almost the minute the first particle of food passed from the stomach into the duodenum. Since London has shown that no absorption occurs until the chyme enters the intestine, our results indicate

19. Fiske and Summer: Jour. Biol. Chem., 1914, **18**, 285.

20. Nencki and Pavlov: Arch. f. Exper. Path. u. Pharmacol., 1897, **38**, 215.

that the very advance guard of amino-acids entering the blood after a protein meal is, in part at least, immediately turned into urea. The interval between feeding and the beginning of urea formation is so short that this conclusion would really be forced on us, even without the Roentgen-ray evidence. Unreasonable as it appears, the organism does not wait until it has absorbed sufficient protein digestion products to meet its immediate requirements, and thereafter begin to turn the surplus into urea. The very beginning of absorption stimulates the urea forming function into activity. This behavior explains the fact that no matter how depleted by disease or hunger the tissues of an individual may be, the greater part of the protein nitrogen which he may subsequently consume is excreted as urea, only a small portion being retained to rebuild the wasted tissues.

That urea is the form taken by all of the amino-acid nitrogen which disappears in the liver does not absolutely follow from our results. Pflüger was of the opinion that the liver cells store reserve protein from the food, just as they store reserve carbohydrate in the form of glycogen. Our results do not at present exclude this possibility. In order to do so, we should have to prove that the liver gives out as urea an amount of nitrogen exactly equal to that which it absorbs as amino-acid, and our experimental technic does not yet enable us to say whether or not this is the case. The analytic methods are adequate, but the fact that the amino-acids are held for a certain time before they are destroyed and that the urea also may not pass instantly from the liver tissue to the hepatic vein makes the striking of an exact balance between amino-acid intake and urea outgo a matter of experimental difficulty which has not yet been overcome. The possibility, therefore, remains open, though certainly not proved, that some of the amino-acids may be converted by the liver into a form of reserve protein which is stored like glycogen.

A word as to the significance of the free amino-acids which are stored as such by the tissues. They normally amount to from 2 to 4 per cent. of the dry weight of the various organs and might be regarded as a form of reserve food. That they are so in the same sense as fat and the glycogen, however, is not the case. The reserve food supplies disappear during a prolonged fast. This occurs with glycogen and fat. It does not occur, even to a slight extent, with the amino-acids. If anything, they are slightly more abundant in the tissues of a fasting animal than in those of one in a state of normal nutrition.<sup>21</sup> I believe that the explanation is that the free amino-acids, in the tissues as in the blood, are merely transitory bodies in either the building up or the breaking down of body proteins. That all the amino-acids in the

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21. Van Slyke and Meyer: Jour. Biol. Chem., 1913, **16**, 231.



muscles of a well-nourished animal may have been immediately derived from food proteins is easily believable. When, however, after a fortnight's fasting we find an equal or greater supply of free amino nitrogen in the muscles, we must attribute the source in this case to autolysis of the visibly disappearing tissues themselves. The free amino-acids are there, both in blood and tissues, because they are intermediate steps in the never-ending processes of the building up and the breaking down of living protein.

We may, perhaps, most easily summarize the facts which have been brought out by tracing an amino-acid through the body as follows: Entering the alimentary tract as part of a protein molecule, it is set free by digestive hydrolysis and passes into the portal blood stream. It may be at once picked up by the liver and decomposed into urea, or perhaps synthesized into reserve protein. It may, however, pass by the liver and be absorbed from the blood by one of the other tissues. Here it may remain for a time before being incorporated into the tissue protein. The fact that a considerable store of amino-acids is always found in the tissues is proof that chemical incorporation does not instantly follow absorption from the blood stream. After a period of time, concerning the length of which we are absolutely ignorant, the tissue autolyzes, and the amino-acid returns to the depot of free amino-acids held by the tissue. From this depot it may pass back into the blood, be taken out by the liver, and destroyed. Or it may be reincarnated into a new protein in some other organ.

We have hitherto dealt with the physiology of the amino-acids without any recognition of the differences between the individual members of the family. Whether we are concerned with their condensation into body proteins or the manner in which, not being so condensed, they are destroyed, the individuality of the different amino-acids plays a most important rôle.

Let us consider briefly the indispensability of the different amino-acids for the nutrition of the body. All of the amino-acids which are known to occur in the native proteins enter into the structure of living protoplasm. The bacterium can synthesize them all from ammonia and sugar. Loeb has recently presented evidence that even lower animals, such as the banana fly, can also synthesize all of their amino-acids.<sup>22</sup> The higher animals can synthesize some, but must be supplied with others. One of the vital questions of physiology today is: "Which amino acids can the mammalian body synthesize for itself, and which must be supplied ready made to it in its food?" The first even partial success in answering this question with experimental evidence was obtained by Hopkins<sup>23</sup> of Cambridge, England. He fed mice with food

22. Loeb, Jacques: *Jour. Biol. Chem.*, 1915, **23**, 431.

23. Hopkins and Willcock: *Jour. Physiol.*, 1907, **35**, 88.

which contained as its sole nitrogenous constituent the corn protein, zein. Zein contains no tryptophan. Eighty per cent. of the mice died within twenty days. When, however, tryptophan was added to the diet, only one fifth of the mice died within twenty days, and most of them lived for over a month. Therefore it appeared, as has since been more rigidly proved, that tryptophan is one of the amino-acids which cannot be made in the body, but must be supplied in the food.

The study of the nutritional function of the individual amino-acids opened by Hopkins' pioneer investigation has been developed by our own chemists, Osborne and Mendel,<sup>24</sup> who have studied the problem with a monumental attention to detail in the care, control, and even breeding of the rats used as experimental animals, in the accuracy with which the chemical composition of the food utilized was controlled, and in the wealth of experimental evidence with which point after point in the field has been settled. Professor Mendel himself has recently

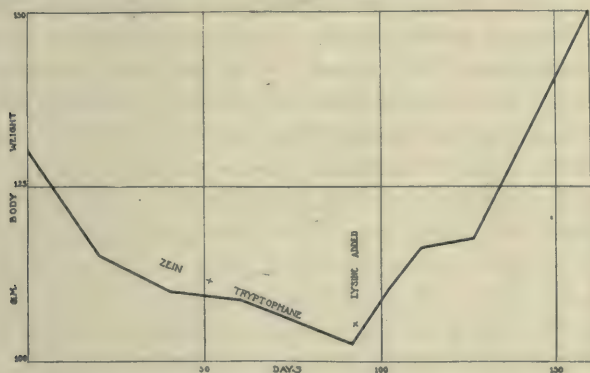


Fig. 8.—Effect of adding lysine and tryptophan to a diet deficient in these amino-acids.

discussed the work before the Harvey Society, and it is mentioned here only because a paper on the physiology of the amino-acids would be incomplete without it. I have reproduced one of the hundreds of curves of growth which Osborne and Mendel have published. This curve forms, in a way, a connecting link between Hopkins' work and theirs. It shows why the mice which Hopkins fed with zein plus tryptophan lived only a little longer than those which received zein alone. The rat whose weight curve is shown (Fig. 8) received at first, like Hopkins' mice, a diet containing zein plus tryptophan as the sole nitrogenous constituents. During this period, although the animal was immature and should have been growing, his weight fell steadily. After ninety days another amino-acid, lysine, was added to the diet. Zein is lacking in lysine as well as tryptophan. The effect of making good both

24. Osborne and Mendel: Publications of the Carnegie Institution.

these deficits is shown by the immediate resumption of practically normal growth. Osborne and Mendel have proved beyond a doubt by such experiments that both lysin and tryptophan must be supplied to the higher animals in their food, since neither is synthesized in the animal body. A third amino-acid in the indispensable class is cystin, and metabolism experiments by Abderhalden<sup>25</sup> indicate that tyrosin is a fourth. That future work will answer the question concerning the synthetic power of the body for other amino-acids may be expected with confidence.

That even the higher animals still retain the ability to synthesize the simplest of the amino-acids, glycocoll, is certain. The excretion of glycocoll can be stimulated by feeding benzoic acid. Instead of neutralizing it with ammonia, as it does with most other acids, the body condenses benzoic with glycocoll to form hippuric acid, in which form it is excreted. Magnus-Levy<sup>26</sup> found that by feeding rabbits large amounts of benzoic acid he could make them excrete more glycocoll in the form of hippuric acid than they possessed, either free or combined, in their entire bodies. This proved that they were able to manufacture glycocoll out of other nitrogenous substances. Osborne and Mendel have also found in their feeding experiments that glycocoll does not need to be fed in order to maintain growth, the rat being able to synthesize the amounts necessary for its growing tissue out of other nitrogenous substances. Whether any of the other amino-acids can be synthesized like glycocoll is uncertain. The field, the enormous importance of which from both the practical and scientific standpoints is self-evident, may be said to be still thirteen-eightieths virgin; concerning thirteen of the eighteen amino-acids we have no conclusive knowledge as to whether we can synthesize them in our bodies or must depend on plants to furnish them for us.

A discussion of the physiology of the amino-acids would not be complete without a word also concerning the manner in which those not incorporated by the body are broken down and in part cast out (as urea), in part burned or stored like fat or carbohydrate for their energy. The three men whose researches entitle them to speak with most authority in this field are Lusk, Dakin and Knoop. Knoop came from Freiburg three years ago to deliver a Harvey Lecture on this subject, and, as our city is fortunate in claiming both Dakin and Lusk we either have heard or may reasonably hope to hear from them both the stories of their own researches. I will, therefore, attempt to indicate only in the most general way the manner in which the body is believed to dispose of its unincorporated amino-acids. The

25. Abderhalden: *Ztschr. f. physiol. Chem.*, 1913, **83**, 444.

26. Magnus-Levy: *Biochem. Ztschr.*, 1907, **6**, 523.



first step is the splitting off of the amino group, which yields ammonia and a hydroxy acid, a hydroxyl group replacing the amino group of the amino-acid. The ammonia is turned into urea. The nonnitrogenous substance left after the amino group is split off from the amino-acid is a fatty acid, and is dealt with accordingly. Varying with their structure, some of the amino-acids yield fatty acids which can be converted into glucose by the body, while others do not. Nearly the entire series of amino-acids has been tested in this respect by either Lusk or Dakin. The substances were either fed to phlorizinized dogs, whose urine was then analyzed for glucose, into which they turn everything that is physiologically capable of being turned into glucose; or the amino-acids were perfused through surviving livers and the perfusion fluid was analyzed for glucose. The results are indicated by the plus and minus signs on the line at the bottom of Figure 3. The fact that half the amino-acids are glucose formers explains why diabetics can form sugar from protein as well as from carbohydrate. The fact that acetone bodies are formed from several amino-acids explains why diabetics may develop acidosis on a protein diet, or even when living on the proteins of their own tissues.

The nature of the fatty acid radicals left when the amino groups are removed from the amino-acids is also used by Lusk<sup>27</sup> to explain the specific dynamic action of the proteins, their ability so to stimulate the metabolism that the rate of heat formation in the body is accelerated. The amino-acids themselves cannot be responsible for this effect, because their concentration in the body is so well regulated, presumably by the liver, that no great fluctuations ordinarily occur, even after heavy consumption of protein. The stimulated heat production which Lusk and DuBois have discovered after the feeding of either protein or of amino-acids must, therefore, be due to their decomposition products, presumably the fatty acids that are formed by deamination, and the differences in the heat-stimulating effects of the different amino-acids are due to their individual differences, as indicated by the varying shapes of their structural heads.

#### PATHOLOGY OF THE AMINO-ACIDS

We now come to the significance of the amino-acids in pathology. The blood and urine have been investigated in regard to their amino-acid content for the purpose of diagnosing or explaining pathologic conditions which may be divided into two classes:

1. Those in which the normal function of catabolizing the amino-acids is injured. From the view that the liver is especially responsible for the conversion of the excess products of protein digestion into urea,

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27. Lusk: *Jour. Biol. Chem.*, 1915, **20**, 615.

it would logically follow that serious injury to this organ should result in a higher amino-acid content of the blood, and perhaps the urine. Consequently amino-acids have been sought for by a number of investigators both as diagnostic indications and as toxic agents in liver atrophy, in conditions which involve visible injury to the liver, such as toxemia of pregnancy, and in conditions which are presumably accompanied or caused by decreased liver function, of which diabetes is an example.

2. In the second type of abnormal condition in which amino-acid determinations have been called to the aid of the diagnostician, specific ferments are supposed to be formed within the body which are capable of hydrolyzing tissues of an abnormal pathologic nature, thereby forming, either in vivo or in vitro, amino-acids from such tissues. The action of such specific ferments in vitro on the particular tissues toward which their activity is directed constitutes the Abderhalden reaction, which has been of late so largely in the public eye. Dr. Losee of the Lying-In Hospital, Mrs. Vinograd-Villchur and I have devoted nearly a year of time that might otherwise have been valuable to this reaction, and I shall consequently devote a moment to it here.

The Abderhalden reaction is based on the belief that when foreign proteins enter the blood stream the body cells elaborate and pour into the circulation enzymes which are capable of hydrolyzing the invading protein and none other. This idea was extended to include the proteins of abnormal tissues produced within the body itself. Thus, the epithelial cells of the placenta of a pregnant woman are supposed to wander into the blood stream and thereby stimulate the production of enzymes which can hydrolyze only the proteins of placenta tissue. Similarly, cancer cells are supposed to cause the production of enzymes capable of hydrolyzing only cancer tissue. The idea has been extended by various investigators to such an extent that, to judge from the claims made for the Abderhalden reaction, all that is necessary in order to settle a difficult diagnosis is to mix a little of the patient's serum with samples of tissue from all the suspected organs of the body, and the serum will infallibly pick out and digest the tissue from the affected part, leaving the other tissues unaltered. In justice to Abderhalden, it must be stated that his claims have never been so sweeping as those of some of his satellites.

A great controversy arose over the Abderhalden reaction, some investigators reporting their results with enthusiasm, while others failed entirely, and still others took a middle course and utilized the customary safety-first formula to the effect that there was evidently something in the reaction, but that results must be accepted with caution. It appeared to us that the matter might be settled decisively if, instead of the rather uncertain color reaction with



ninhydrin to detect the amino-acids resulting from digestion of the specific tissue, an accurate quantitative method were applied. And the nitrous acid reaction because of its combined accuracy and specificity for amino groups, seemed to offer par excellence such a method. After preliminary experiments to ascertain the most satisfactory way in which to apply it, we finally settled on the following simple technic. Two c.c. of serum are incubated with placenta, as described by Abderhalden, and the undigested proteins are then removed by precipitation with colloidal iron. A control portion of serum is incubated and precipitated in the same way, but without placenta. The amino nitrogen is then determined in both protein-free filtrates. The increase in the nitrogen gas from the serum plus placenta over the nitrogen from serum alone indicates the extent of digestion that has taken place. The results could be obtained with great accuracy, and the increases observed were many times larger than the experimental error. Consequently we believed that we were successful in excluding such error as a factor in interpreting the results. In order to give the reaction the fairest test possible we utilized it only as a test for pregnancy, and the controls were normal men and women, not nonpregnant hospital patients. We made several hundred analyses, but the nature of all the results is completely indicated by Figure 9. Both normal serums and serums from pregnant women show a measurable amount of digestive activity, and the results with both vary over practically the same range. A slightly higher average obtained with serums from pregnant women may explain the fact that some honest investigators have been led to believe that if they could eliminate their own errors they would find the reaction all that had been claimed for it. But even the difference in averages is not significant, and the individual results from perfectly normal subjects cover practically the same range as those from pregnant women.<sup>28</sup> Entirely similar results were obtained by Isaac Levin and myself<sup>29</sup> in attempting to apply the reaction to cancer diagnosis.

We finally come to attempts to detect by amino-acid determination conditions involving impaired liver function. That tyrosin may be found in the urine in acute yellow atrophy is the classical fact in this field. The more the problem is studied with quantitative methods, the more it appears, however, that the liver injury must be extreme before it can cause unusual accumulation or excretion of amino-acids. Soon after the nitrous acid method was perfected I determined the amino nitrogen in the urine of dogs which Drs. Dochez and Opee had treated with chloroform and phosphorus, and was greatly surprised to find no

28. Van Slyke, Losee, and Vinograd: *Jour. Biol. Chem.*, 1915, **23**, 377.

29. Levin and Van Slyke: *Jour. Am. Med. Assn.*, 1915, **65**, 945.



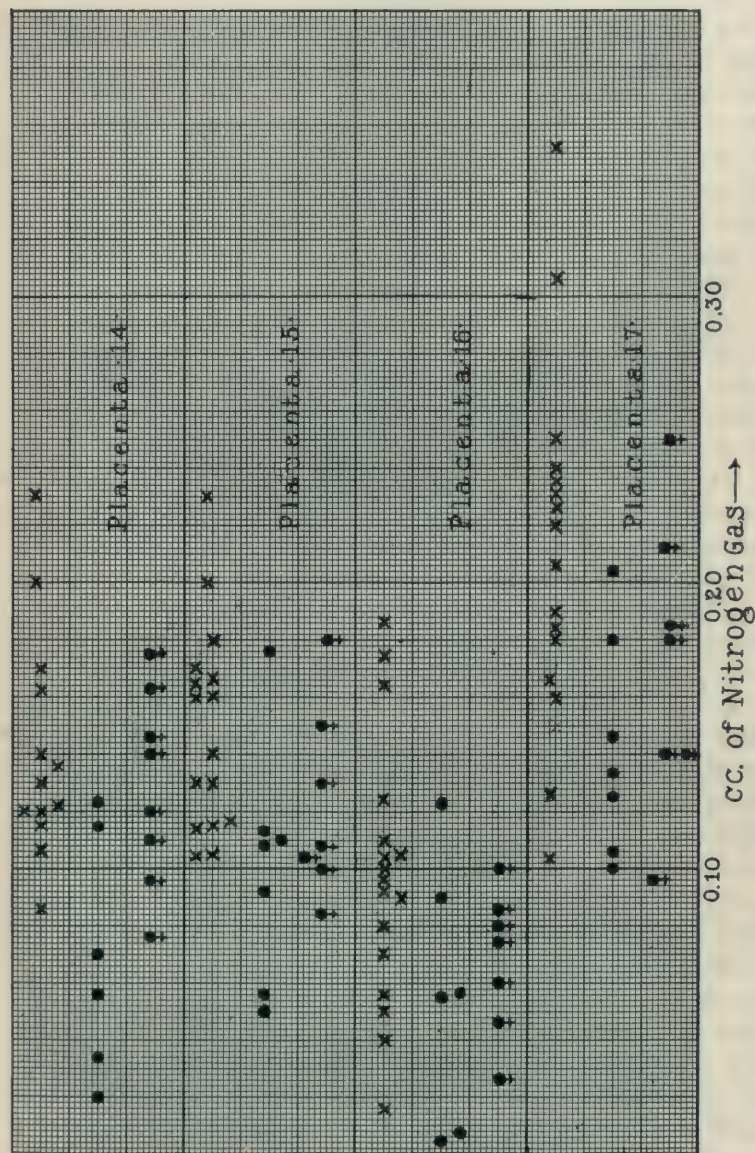


Fig. 9.—Quantitative results with the Abderhalden reaction. The abscissas represent values of the difference (cubic centimeters of nitrogen from 2 c.c. serum incubated with placenta minus cubic centimeters of nitrogen from 2 c.c. serum incubated alone); that is, the abscissas give in terms of amino nitrogen the extent of protein digestion caused by the interaction of serum and placenta. The results from normal male sera are indicated by ●; the results from normal female sera are indicated by ○; and the results from pregnant sera are indicated by X. The results obtained with each placenta are grouped between a pair of horizontal lines, each cross or circle representing the result obtained with the serum of one pregnant or nonpregnant individual acting on the placenta indicated.

increase in the percentage of amino nitrogen, despite the fact that necropsy showed extreme liver degeneration. More recently Marshall and Rowntree<sup>30</sup> have found that the urine of such dogs, if taken immediately before death occurs, does show an increase in amino nitrogen, and that a still greater increase occurs at this time in the blood. It is evident, however, that in dogs at least the liver injury must be most severe in order to affect the amino-acid content of the blood or urine. That it must be equally severe in man does not necessarily follow. Chesney, Marshall and Rowntree<sup>31</sup> report that a considerable proportion of patients with impaired liver function showed abnormally high amino-acid nitrogen in the blood. Consequently, although it cannot be said that the amino nitrogen determination in either blood or urine offers at present much assistance to the diagnostician of diseased livers, it may be possible that the very constancy of the amino-nitrogen figure under almost all conditions will enhance its diagnostic value for such conditions as do affect it.

That advanced diabetes is such a condition has recently been claimed by Cammidge.<sup>32</sup> According to his view in the milder stages of diabetes the body partially loses its ability to burn glucose, but it can still transform amino-acids into glucose. In the most severe stage, however, it cannot even accomplish the preliminary transformation of amino-acids into glucose, but excretes large amounts of them unchanged. We have performed determinations of amino nitrogen in both blood and urine on a considerable number of patients in Dr. Allen's diabetic clinic at the Rockefeller Hospital and have found that urine from certain of the patients does show figures distinctly higher than normal. That the high figures indicate diabetes of a special gravity, however, we are not yet prepared to state.

We finally come to the toxemias of pregnancy. Ewing and Wolf<sup>33</sup> some years ago showed that the urines in such cases had a decreased proportion of urea nitrogen and an increase in the undetermined nitrogen. From this, and from the gross injuries which the liver suffers during the toxemia, Ewing and Wolf suggested that the intoxication might be due to protein digestion products which the degenerated liver could not desamidize, and which caused both the toxic symptoms and the increase in the undetermined nitrogen of the urine. The methods used for urine analysis were the most complete available at the time the work was done, and the hypothesis put forward was certainly reasonable. However, Dr. Losee and I, in examining both the urine and the blood from a considerable number of patients with toxemia of

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30. Marshall and Rowntree: *Jour. Exper. Med.*, 1915, **22**, 333.

31. Chesney, Marshall, and Rowntree: *Jour. Am. Med. Assn.*, 1914, **63**, 1706.

32. Cammidge: *Lancet*, London, 1913, **2**, 1319.

33. Ewing and Wolf: *Am. Jour. Obst.*, 1907, **55**, 289.



pregnancy, have found in no instance that either blood or urine showed an abnormal concentration of amino-acids or of intermediate protein digestion products. Consequently, the responsibility for the toxemias of pregnancy cannot be left with the amino-acids. I may add that we have also tested the hypothesis that acidosis is to blame, with essentially negative results. We must frankly face the fact that we are entirely ignorant concerning the chemical nature of the substances which cause these toxemias.

I must acknowledge the debt to my collaborators, without whom a large part of the work would have been impossible. I refer to Dr. Gustav Meyer, who collaborated in all the work thus far published, and to Cullen and McLean, members of the Rockefeller Hospital staff, to whose efforts are due results that have been reported for the first time this evening.

It is furthermore a pleasure, as well as a duty, to acknowledge my indebtedness to Dr. Levene, for six years my chief at the Rockefeller Institute. The work detailed this evening is a direct outgrowth of Levene's own researches on the proteins, was carried out with the constant inspiration of his enthusiasm, and help of his counsel, and of his generosity in making available every facility which the laboratory afforded, even at times to the delay of his own immediate work, the ultimate sacrifice that can be taken from a spirit such as his.



## PROGRESSIVE MUSCULAR DYSTROPHY AS AN ENDOCRINE DISEASE \*

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In the present paper, the author desires to report a somewhat atypical form of progressive muscular dystrophy, rather resembling Erb's infantile type, of extremely benign and slow progress. In the cases examined it has occurred as a hereditary affection now in the fourth generation. The number of individuals disabled thus far has been fourteen. Of the seven living members with the disease, all but two have been examined by Roentgen ray, and of these five, four show distinct changes in the pineal gland, producing shadows in the roentgenogram. The evidence presented by the cases reported in the present paper, together with that of previous communications in this field, creates a strong probability of the existence of a close relationship between progressive muscular dystrophy and disease of the pineal gland.

In order to understand the evolution that has now placed this disease among the endocrinopathies, a rapid survey of the development of the syndrome, together with the most important contributions thereto in the last few decades, is almost indispensable and is herewith included. The cases studied have been placed at my disposal by Dr. Pearce Bailey, of the Neurological Institute, and for his valuable suggestions and actual assistance, I desire to express my appreciation and my thanks.

*Historical.*<sup>1</sup>—The first observations on muscular atrophies are found in Boerhaave's Aphorisms (1709), in which Van Swieten mentions the involvement of the deltoid and that of the intrinsic hand muscles. Later, Charles Bell described syndromes including progressive muscular atrophy. But it was reserved for Aran and Duchenne in 1850 to give the first good description of progressive muscular atrophy. Following them, the French physicians called this type of disease "atrophie musculaire progressive type Duchenne-Aran." Shortly thereafter, Cruveilhier, on the basis of a necropsy, stated that the cause of the disease lay in abnormality of the spinal gray; and ever since then the discussion

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1. Müller, Albert: *Zur Lehre der Dystrophia Muscularis progressiva*, Dissert., Kiel, 1902, Surgeon General's Library.

has waxed hot as to whether the lesion is in the spinal cord or in the muscles themselves. A third theory explained it as a trophoneurosis (Remak, Schneevogt, Jaccoud), with disease of the sympathetic system as a basis. This idea was soon given up. As histologic technic improved and positive findings in the spinal cord increased, the supporters of the myelopathic character of the disease seemed to carry the day (Luys, Hayem, Jaffroy and especially Charcot). Charcot, as a result of many investigations, declared the large multipolar ganglion cells of the anterior horns to be the trophic centers of voluntary muscles and that in their alterations the cause of atrophic muscle disease was to be found. Duchenne himself, although originally arrayed on the side of the myopathic nature of the trouble, came around to Charcot's view. So likewise Erb. In spite of the fact that most neuropathologists agreed with Charcot, Friedreich opposed him. In his monograph<sup>2</sup> of 1873 the latter defended the myopathic nature of the trouble in many cases. Thus, he stated that as a result of successive extreme exertions in the same muscle groups, especially in a constitutional diathesis weakening the muscular system's resistance, the atrophies occurred. He described cases following shortly after typhus, typhoid, measles, acute rheumatism, cholera and even after the puerperium, and thereupon declared the condition to be one of "polymyositis chronica progressiva." The changes in the anterior horn cells he considered secondary to the muscle deterioration. But the majority of neurologists stood behind Charcot. Then in 1878 Lichtheim presented a case with high-grade atrophy of great extent in which no cord changes were seen at all, nor were any evident in the peripheral nerves. Therefore, he came to the necessary conclusion that changes in the anterior horn cells are not a *sine qua non* of the condition, and further, that Charcot's theory could not be the true one. Charcot's preeminence, however, still carried the day. Finally, more recent investigators, after carefully separating all conditions that resembled the progressive muscular atrophies, such as poliomyelitis, chronic multiple neuritis, atrophies with joint lesions, syringomyelia, and so on, from them, two distinct types remained; those that did show spinal cord changes, and those that did not. In 1876 Leyden proposed to separate hereditary forms, on account of their etiology and the localization of the atrophies (pelvic girdle), from the Duchenne-Aran type, and at the same time he showed their resemblance to the hypertrophic type. Lastly, Erb, in 1882, on the basis of a vast variety of cases personally examined by him, declared that there were two distinct types of progressive muscular atrophy, different in their syndrome, localization, evolution, and in their objective behavior, as well as in their anatomic foundation; a spinal

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2. Friedreich, N.: *Ueber progressive muskeltrophie; über wahre und falsche Muskelhypertrophie*, Berlin, 1873.

atrophic form and a muscular dystrophic form. To this differentiation even the Charcot School assented. But even here it did not take long to discover that there were transitional forms, and in each group of cases intrinsic differences. These depend on the different muscle groups first affected, and on the degree and nature of the muscle changes. Thus have arisen the many confusing types of the disease, and not, as at first thought, distinct diseases. One great work which we ought to be thankful to Erb for having done for us is that he grouped all these muscular types in one large family, which he called *dystrophia muscularis progressiva*.<sup>3</sup> In this family he included his juvenile *muskelatrophie*, *pseudohypertrophie* infantile form and the hereditary forms. The spinal types he placed under the name "*Amyotrophia spinalis progressiva*, Duchenne-Aran." Soon thereafter Landouzy and Dejerine (1885) described two cases of "*atrophie de l'enfance*"<sup>4</sup> in one of which they obtained a necropsy. To their great surprise, they found no changes in the spinal cord, and described the case as a new one, differing entirely from the Duchenne-Aran kind. They called it "*myopathie*, type facio-scapulo-humeral," and a second type with "*intégrité de la face*, type scapulo-humeral." Undoubtedly these types are nothing else than the Erb juvenile form, which at times involves the face and again does not.

In the course of time, all observers, Charcot and Marie included, agreed to accept this classification of Erb's:

1. *Dystrophia muscularis progressiva* (infantum).
  - a. Hypertrophic form
    - (1) With pseudohypertrophy
    - (2) With true hypertrophy
  - b. Atrophic form
    - (1) With primary facial involvement (Duchenne's infantile form)
    - (2) Without facial involvement. Simple atrophic form.
2. *Dystrophia muscularis progressiva* juvenum et adultorum.  
(juvenile form)

It is characteristic of the familial, hereditary forms to exhibit conditions at times of one of these groups, at others, of another group; so that in several generations of the same family individuals will be found to conform with almost any one of the types outlined above. It is so with the family herein studied.

#### TYPES OF THE DISEASE

Various authors have described atypical forms of muscular dystrophy, that is, they were atypical either in their course or their chron-

3. Erb, W.: Ueber die juvenile form der progressiven Muskelatrophie, *Deutsch. Arch. f. klin. Med.*, 1884, **34**, 467.

4. Landouzy, L., and Dejerine, J.: De la myopathie atrophique progressive, *Rev. de méd.*, 1885, **5**, 81.



icity, or else in the groups of muscles affected or in the intensity of such impairment; in the presence or absence of contractures; in the slow or rapid involvement of the entire skeletal structure, together with the muscular, making of the patients helpless cripples, and ending in death in a comparatively short time. Some of these important types it may be well here to describe and classify, so that their important characteristics may serve to differentiate them at the same time from the group of cases which I propose to submit further on in this paper. It will also be noticed that as we progress the recent authors show more and more marked a tendency to include symptoms referable to the sympathetic nervous system, including the endocrine glands.

Barsickow's group is late in appearance, slowly progressive and not fatal. It was the first group of great importance, studied by Barsickow<sup>5</sup> in 1871, and consisted of twenty-four cases occurring in two families from one ascendent through five generations. This series resembles most closely the series herewith to be presented, and yet differs from it in noteworthy particulars. It is presented in extenso for its importance. A composite picture of Barsickow's cases showed that the members of the widely scattered families in which they arose were as a rule in good health and lived to be old. The greatgrandfather had only a stiffness in gait and carriage, and yet twenty-three of his descendants (five out of seven of his immediate children) showed muscular disease, not only in function, but also in muscle volume. The members seem to have been attacked without rule, seemingly showing some predisposition in heredity. The children remained healthy if the parents were unaffected, and there were an equal number of males and females attacked. The cause of the original incidence, Barsickow states, was probably lead poisoning, for this original grandfather was a typesetter and had had many attacks of lead colic. In some cases chlorosis, cholera and varioloid acted as preceding agents conducing to the development of the trouble. The onset in seventeen of the cases was at the following ages: five between the ages of 10 and 20, seven between 20 and 30, and five later in life.

It was ushered in by almost simultaneous affection of both an upper and lower limb, or both upper and lower limbs. The serratus anticus was among the first muscles to cause trouble. The most prominent symptom was the disturbed muscular function; in the mildest cases being stiffness and rapid exhaustion, going to absolute paralysis of single muscles, or entire groups in severe cases. The involvement was symmetrical. In most of the cases the diseased muscles gradually disappeared or atrophied, and hypertrophy was very rare. Frequently there was a lordosis with winged scapulae, with protrusion of the

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5. Barsickow, Hermann: *Zwei Familien mit Lipomatosis muscularis progressiva*, Dissert., Halle, 1872, Surgeon General's Library, Washington.

shoulders or flattening of the chest. Fibrillary twitching was rare, only once in fact. There was in the skin occasionally loss of pigment or abnormal coloring. Of the eleven who died while under Barsickow's observation, one was over 80 years of age, four over 70 years, three over 60, one 58 and two 33. So that the disease apparently did little to cut short their lives.

Taking up Barsickow's individual cases, those that showed symptoms seemingly not accounted for in the usual syndrome of *dystrophia musculorum progressiva*, we see in his Case 1 the complaint of rheumatoid pains in many parts of the body, dirty brown pigmented skin and patches of vitiligo. Case 19 was that of a woman of 40 in whom the fingers and toes easily fell asleep and got cold, white and waxy in the winter. Case 7 was that of a woman in whom the disease began at the menopause.

The reason I mention the above symptoms, which in the light of our present knowledge are referable to a disturbed autonomic nervous system with inclusion of the endocrine glands, is that the theory of Remak, Schneevogt, Jaccoud, and Dumenil relative to the dependence of the affection on such disturbance of the vegetative system has now been shown to have some foundation.

The microscopic examination of the muscle fibers in Barsickow's cases showed changes in the muscle fibrils, some of which were much thickened, while between the primitive muscle bundles were many fat cells and connective tissue. The skin spots appeared in the neighborhood of recently affected muscles. In three cases with necropsy there was no abnormality of the nervous system demonstrable, and yet the author inclined to the idea that the disease is of a "vasomotor-trophic-nerve character." The Barsickow cases undoubtedly belong to the Erb juvenile and adult types.

In Friedreich's group of cases the disease was early in appearance, rapidly progressive, and fatal. Friedreich<sup>6</sup> published the cases of four brothers whose mother was unaffected by the disease, but two of whose maternal uncles died of it in their 15th and 16th years. The brothers cited died at 5, 6, 12 and 16 years of age. Intellectually they were all well advanced. The affection began in the weakness of the lumbar muscles, with constantly increasing difficulty in arising from a sitting posture. Muscular atrophy began in the muscles on each side of the spine at the same time. There then arose a difficulty in raising the arms to the shoulders on both sides. One of the victims died of suffocation from an asthmatic bronchial attack. In this series of cases the rapidity of the course to a fatal termination is the characteristic. The necropsy showed, among other atrophies, that the pectorals were

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6. Friedreich, N.: *Progressive Muskelatrophie*, Berlin, 1873.



reduced to thin grayish-red, skin-like lamellae, through which the ribs showed.

In Gowers' group the disease was early in appearance, rapid in progress, and was fatal in adolescence. Gowers<sup>7</sup> in a clinical lecture in 1879 reported four brothers, aged 9, 10, 5 and 3 years, all affected, whose parents were both healthy, but whose maternal uncle died at 15 of a wasting muscular disease. These patients had a little difficulty in putting their feet down on the floor on account of a tense, shortened Achilles tendon. The extensors of the knee and hip were weak; the flexors of the hip were feeble. The latissimus dorsi and the lower part of the pectorals were gone. There were no sensory changes, the kneejerks were absent and there was a marked lordosis. The upper limbs were weak, although they moved freely. Gowers reports that out of 220 cases that he collected from the literature, 190 of the patients were male and thirty were female. While he mentions Barsickow's cases, yet he excludes them from his statistics, because they were all of the adult type. Some generalizations from these 220 cases are drawn by Gowers: (1) the disease almost never is heard of on the side of the father; (2) the age of onset is an etiologic factor of great importance and occurs in the worst cases before the 6th year, the more severe the case, the earlier it begins; (3) occasionally the disease follows physical injury; (4) shortening and permanent contractions of certain muscles lead to distortions in the positions of the joints; especially contraction of the calf muscles, leading to inability to place the heels on the floor; (5) the patients lose the power of standing at 10 or 12 years of age, and death comes on between 14 and 18 years; (6) of diagnostic importance is not the actual muscular enlargement, but the distribution of the muscular disease, especially the wasting of the lower pectorals and the latissimus dorsi; (7) the later the appearance of the disease, the more slowly it advances; the older the patient, the better the prognosis.

The commentary on these cases is that some of the conclusions, especially the last, are quite contrary to our type of the disease to be described hereinafter.

Erb's<sup>8</sup> types are of the accepted classification of progressive muscular dystrophy. In one of the publications of Erb<sup>8</sup> he gives a general description of the juvenile type of the disease, saying that it begins always before the 20th year and usually with atrophy of the upper arms and shoulder girdle muscles and is often combined with hypertrophy. There are no reactions of degeneration and no muscular fibril-

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7. Gowers, W. R.: Pseudohypertrophic Paralysis, Clinical Lecture, London, 1879.

8. Erb, W.: Dystrophia musculorum progressiva, Deutsch. Ztschr. f. Nervenhe., 1891, 1, 173.



lary twitching. The following muscles are almost constantly affected; pectorals (except the clavicular portion), cucullaris, latissimus dorsi, biceps and brachialis anticus and the long supinator. The glutei are weak and calves large. The disease lasts from 20 to 40 years with periods of quiescence. He states that this juvenile form is identical with Friedreich's hereditary progressive muscular atrophy. These latter forms belong in the same group with pseudohypertrophy.

In one publication,<sup>8</sup> Erb gives the results of the examination of the available pathologic material of all these forms and says in summarizing that all the noticeable changes are in the muscle fiber chiefly, from an increase in volume to absolute disappearance of the muscle. There is an increase in the muscle nuclei and these are both normally and abnormally placed. There are clefts in the long axis of the muscle between the muscle fibers and an increase of connective tissue is found with a later deposit of fat cells and a thickening of the vessel walls. As a result of the identical pathologic findings in all forms of this elusive muscle disease, he proposes the classification of all these forms as types under the generic term of *dystrophia musculorum progressiva*. This classification has already been given earlier in this paper.

Among other types the next cases of importance in the development of the semeiology of the disease were reported by Prager.<sup>9</sup> Here the patients, two in number, were children of first cousins, who themselves were free of the disease. The importance of the citation of one of these cases is in the fact that while the patient, a woman of 48, had always had a waddling gait and a difficulty in going upstairs, yet after her first puerperium, which lasted three months, the difficulty in walking was so intensified that she needed support. Her muscular system gradually underwent the usual atrophies in the pectorals, latissimi, trapezius, rhomboids, biceps, deltoids, triceps and supinator longus; but these atrophies were disguised by masses of fat in the arms and legs. To these physical signs, however, were appended the statements: (1) the patient was easily excited and then developed a tremor; (2) there was difficulty in deglutition for fifteen years; (3) dyspnea in cold weather and (4) increase in stools. Further on is the remark that the patient had a "struma" on the neck and complained of urticaria. That is, she was a hyperthyroid subject. We shall find as we go along in the analysis of the symptoms as presented by the author frequent indications of disturbance in the endocrine system. In all probability, had such indications been then understood, there would have resulted a far wider range of symptoms in the muscular dystrophies; for what to the older observers probably appeared negligible in this regard, now assumes great importance. We shall see how more and more often

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9. Prager, M.: *Dystrophia musculorum progressiva*, Dissert., Erlangen, 1891.

in later years investigators have described conditions present in progressive muscular dystrophy, apart from the actual muscle changes, that are seemingly part of the disease process; which conditions have received but scant reference in the earlier works, presumably because they seemed so utterly adventitious. So Hahn<sup>10</sup> states that skeletal anomalies are often seen and the idea is now that these are part of the condition of progressive muscular dystrophy; and he cites Friedreich that bone atrophy is not secondarily due to disease of the bone through immobility of the joint, but to nervous and trophic influence. Eulenberg<sup>11</sup> had a case which was combined with acromegaly. Hahn's conclusion is that because so many cases show bony changes, there may be some connection between the two.

Bregman<sup>12</sup> cites cases in which together with a "facies myopathique" there was difficulty in looking up and in closing the eyes; inequality of the pupils and of the palpebral fissures. This is interesting from the fact, to be shown from our own cases, of the frequent involvement of the pineal gland; in which condition just such eye muscle difficulties arise (Bailey and Jelliffe<sup>13</sup>). Bregman's cases further showed internal glandular disturbances as follows: In Case 1 the patient could not close the eyes properly and had nyctalopia; also there was intense sweating of the extremities with cyanotic hands and a pulse of 100. In Case 2 there was marked prognathism, the upper teeth standing prominently forward; the skin was mottled like marble. In Case 4 the hands and feet were livid; there was large skeletal development of the hands and feet in contrast to the rest of the skeleton, and marked protrusion of the upper jaw. In Case 5 there was extremely large body growth. In these cases there is manifestly disturbance of both pituitary and thyroid glands.

Cestan and Lejonne<sup>14</sup> publish two cases with contractures and state that it is banal to say that contractures accompany all forms of progressive muscular dystrophy, but that ordinarily contractures are slight and rarely sufficiently marked to alter the general attitude of the myopathic patient. Schultze<sup>15</sup> presents two cases, both with necropsy, of brother and sister; in the former there was a thinning of the long bones, the humerus being thinner than the middle finger of a normal hand, and

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10. Hahn, F.: Ueber d. Auftreten v. Contracture bei Dystrophie, Deutsch. Ztschr. f. Nervenhe., 1901, **20**, 137.

11. Eulenberg: Deutsch. med. Wchnschr., 1896, **22**, 458.

12. Bregman, L. E.: Ein Casuistischer Beitrag z. progressive Muskelatrophie, Deutsch. Ztschr. f. Nervenhe., 1899, **14**, Cases 3 and 4.

13. Bailey, Pearce, and Jelliffe, S. E.: Tumors of the Pineal Body, THE ARCHIVES INT. MED., 1911, **8**, 851.

14. Cestan and Lejonne: Une myopathie familiale avec rétractions, Nouvelle Iconograph, d. l. Salpêtrière, 1902, **15**, 38.

15. Schultze, Fr.: Ueber Combination v. famil. progress. Pseudohypertr. d. muskeln m. Knochenatrophie, Deutsch. Ztschr. f. Nervenhe., 1898-1899, **14**.



the medullary cavity very small. The sister had a stiffness in all the joints of the body, with atrophy of the bones. Schultze states that he had found only two other instances in the literature in which was found bone atrophy with muscular dystrophy, one a case of Friedreich's and the other Le Gendre's.<sup>16</sup> In all these cases the atrophy was a concentric one of the long bones with no diminution in the length. In Le Gendre's case there was also an undue hypertrophy of the genitals, with an enlarged prostate gland in a youth aged 20; while Friedreich's case showed infantilism in the sexual organs, voice and facial expression. The necropsies in Schultze's cases showed no apparent changes in the spinal cord, not even in the ganglion cells, and he therefore concludes that we must look elsewhere than in the nervous system for the cause of this "riddle-like disease," and advises us that we cannot neglect the theory of predisposition, with accidental factors superimposed, such as overstrain, trauma, underfeeding, infection and intoxication.

O. With<sup>17</sup> published a familial type of the disease affecting three boys in one family and sparing the four girls. What interests us in these cases is the involvement of the tonsils, which were hypertrophied, a chronic angina, a hypertrophied lower jaw, with difficulty in mastication and deglutition.

An article of extreme importance on account of the cure of the patient, by Marina,<sup>18</sup> describes a case of a girl of 8½ years of age, who had all the signs of a beginning dystrophy with hypertrophic deltoids and gastrocnemii and so weak in the shoulder girdle group that she could barely lift her arms horizontally. He lost sight of her for five years and then she appeared again entirely well and cured. The author corresponded personally with Erb in this matter, questioning him as to whether a patient with muscular dystrophy had ever in Erb's experience been cured, or whether he had ever seen "formes frustes" of this disease. Erb replied that he had never seen a single case of "formes frustes"—they had all been progressive—but that he had seen one single case of cure in a young English girl. Erb and Marina agreed that the cure in both of these cases was to be credited to the normal evolution of the patient; presumably to the fact that following the beginning of menstruation, normal development of the internal secretion succeeded, a most pregnant idea in the light of our present knowledge of the interrelation of the ovaries with the other endocrine glands. A fitting companion piece to the above is the case of Levi and Rothschild<sup>19</sup>

16. Le Gendre: *Gaz. méd. de Paris*, 1860, **15**, 365.

17. With, Otto: *Eine familiäre atypische Form d. Dystroph. muscul. prog.*, Dissertation, Freiburg, 1906, Surgeon General's Library.

18. Marina, Alessandro: *Gibt es Formen Frustes d. muskulären Dystrophy?* *Deutsch. med. Wchnschr.*, 1908, **34**, 1087.

19. Levi and Rothschild: *Rev. neurol.*, 1907, **15**, 613.



of a myopathic atrophy which showed considerable improvement on pituitary extract.

Henri Claude<sup>20</sup> in 1908 described a case of familial progressive muscular dystrophy of rather different character from the usual in that the patient had asymmetrical atrophies, more marked on the right side. Besides the muscular condition, the bones on the right side were less developed than on the left side, as shown by roentgenogram, and there were vasomotor changes particularly evident in the right hand, with perspiration and cyanosis. In cold weather the patient could not use this hand on account of the disability thereby occasioned. The temperature of the right hand was constantly lower than the left and the sphygmomanometric pressure was also less on the right side. Claude drew the conclusion from these findings that the whole disease picture does not embrace merely the muscular difficulty, but includes as well the central or peripheral nervous systems.

Jendrassik<sup>21</sup> after citing the cases of Marina and Erb, above mentioned, in which a seemingly spontaneous cure had been accomplished in muscular dystrophy coincident with adolescence, presents two somewhat similar cases, of which the one was a true progressive dystrophy. This patient was a girl who, just before puberty set in, had had the dystrophic process cease, and then after menstruation had been established became entirely cured, even to the return of the knee reflexes. There had been a precocious body growth between the 10th and 11th years, in which the breasts had participated, growing to a mature size.

Von Werdt<sup>22</sup> describes a case with necropsy, which arose in a woman at the advanced age of 46 and was not familial. It came on after a first pregnancy complicated with phlebitis. She became immediately bed-ridden, and remained so until her death at 63 years. The postmortem examination showed, besides the usual muscle condition, changes in the thyroid, which had developed a large colloid struma with but a little functioning tissue, a small tumor in the spleen, softened suprarenals, small pancreas, somewhat atrophic ovaries and small fatty deposits in the liver. This case is interesting and suggestive as having developed after a disturbance of the ovaries due to a late first pregnancy.

Boveri<sup>23</sup> described several members of a family afflicted with muscle dystrophy in which some of the muscles had been entirely replaced by fibrous tissue or fatty tissue, and thought it remarkable

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20. Claude, Henri: *Dystroph. muscul. progressiv. familial*, *L'encephal*, 1908, Part 2, p. 512.

21. Jendrassik, Ernst: Gibt es heilbare Fälle von Dystrophie? *Deutsch. med. Wchnschr.*, 1909, **35**, 830.

22. Von Werdt, Felix: Ein Fall v. *Dystroph. musculor. progressiva m. Sektionsbefund*, *Ztschr. f. Pathol.*, 1908-1909, **2**, 577.

23. Boveri, P.: *Nevrite hypertroph. familiale*, *Semaine méd.*, 1910, **30**, 145.

that all of the patients had also exophthalmic goiter. Collins and Climenko,<sup>24</sup> among their numerous cases, make mention of the general growth anomalies in several, apart from the general muscular dystrophy. Thus, one patient had a general adenitis with abnormal teeth, especially the incisors, and undescended testicles. Another one had spongy gums, poor and irregularly placed teeth, a high arched palate, long uvula with large tonsils; at the age of 14 this patient was undersized and had as yet no signs of puberty.

Other authors report the presence of anomalies in the skull with asymmetry. Seegard in 1905, on the basis of twenty-one cases, disputed the hereditary factor in the etiology and claimed the entire process to be a metabolic one.

M'Crudden and Sargent,<sup>25</sup> in a careful laboratory study of a case of progressive muscular dystrophy, showed that a condition of hypoglycemia probably underlay the great muscular fatigability. On account of the close relationship between hypoglycemia; muscular asthenia and deficiency of the suprarenals and hypophysis, epinephrin and pituitary extract were administered with resulting improvement in health, strength and weight.

#### DISCUSSION OF PATHOGENESIS

We see that the more recent investigators have severally and individually been approaching a position regarding progressive muscular dystrophy whose supports are disturbances in the endocrine glands, and have begun to consider the actual muscular disturbances merely as incidents in a widespread affection. And indeed, when we consider the converse of the proposition, namely, that known endocrine glandular disturbances, especially those of the pineal gland, have produced in the muscular, bony, and vasomotor systems conditions very similar to those found in the muscular dystrophies (although not all at one time in any one patient), we must accept some causal relationship between the two. It may be here not amiss to cite, among many observations, some of those that bear directly on this latter proposition. First and foremost is the contribution of Bailey and Jelliffe<sup>18</sup> on tumors of the pineal gland. In their own case, one of the prominent symptoms, and one for which the patient originally sought relief, was difficulty in walking. The several examinations disclosed a hypotonic condition of the muscles of the legs. Furthermore, the patient had grown fat rapidly. In some of the cases appearing in the literature of pineal gland tumors, cited by Bailey and Jelliffe, symptoms and signs

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24. Collins and Climenko: Clinical Study of Fifty Cases of Muscular Dystrophy, Post-Graduate, 25th Anniversary Number, 1908.

25. M'Crudden and Sargent: Hypoglycemia and Progressive Muscular Dystrophy, THE ARCHIVES INT. MED., 1916, **17**, 465.

are disclosed that are interesting to us now in the light of the findings in progressive muscular dystrophy. We quote some of these. Massot's<sup>26</sup> patient complained that his leg could not support him. Feilchenfeld's<sup>27</sup> patient showed great weakness of the lower extremities with much loss of motor power. One of Nothnagel's<sup>28</sup> patients had a very marked disturbance in gait of a waddling character and the general bodily musculature was weak, and although the man was large and seemingly strongly built, he had much fat deposited throughout.



Fig. 1.—Attitude at rest, standing, showing the contractions at the elbow and phalangeal joints; the disparity between fairly normal calves and atrophied thighs; also the leaning forward of the head and the retraction of the chest.

26. Massot, M.: Note sur un Cas de tumeur cerebrale avec polyuria, Lyon méd., 1872, **10**, 373.

27. Feilchenfeld, L.: Ein Fall v. Tumor cerebri, Neurol. Centralbl., 1885, **4**, 409.

28. Nothnagel, H.: Geschwulst d. Vierhügel, Hydrocephalus abfließen von cerebralflüssigkeit durch die Nase, Wien. med. Bl., 1888, **11**, 162.



Of interest is Zenner's<sup>29</sup> patient, who developed marked contractures in both arms. Von Hoesslin<sup>30</sup> describes a patient with pineal tumor, who, although he had no paralysis, did develop extreme weakness of the lower extremities so that they could no longer support him. Hempel<sup>31</sup> observed a patient who developed general muscular weakness, and later became bedridden on account of contractures in the extremities, so that he was almost unable to move hand or foot. He lay in bed all drawn together. Joukovsky<sup>32</sup> describes an infant of 6 days with pineal cyst who had contracted arms. Marburg's<sup>33</sup> patient



Fig. 2.—Manner of arising from the floor; climbing up on thighs.

29. Zenner: A Case of Tumor of the Pineal Gland, *Alienist and Neurol.*, 1892, **13**, 470.

30. Von Hoesslin, R.: Tumor d. Epiphysis Cerebri, *München. med. Wchnschr.*, 1896, **43**, 292.

31. Hempel, K.: Ein Beitrag zur Patholog. d. Glandula Pinealis, *Inaug. Dissert.*, Leipzig, 1901.

32. Joukovsky, V.: Hydrpcephalie et tumor d. l. glande pinéale, *Rev. mens. d. mal. de l'enf.*, 1901, **19**, 197.

33. Marburg, O.: Zur Kenntniss d. Histologie d. Zirbeldürse, *Arb. a. d. Neurol. Institut*, 1909, **17**, 217.

developed much fat and showed weakness in one upper extremity and diminished motor power in both lower extremities. Hart<sup>34</sup> published a case in which among the prominent symptoms was increasing muscular fatigability.

Raymond and Claude<sup>35</sup> reported a case most apropos of a glioma of the pineal gland in a boy of 10. He began to grow fat, and grew weak rapidly. Then the left leg became feeble, so that he could scarcely raise it from the ground. He then developed much muscular weakness and finally contractures in the neck and arms arose. Here



Fig. 3.—The isolated muscle bundle that still remains of the left pectoralis, the rest of the muscle being gone; note the funnel-like neck sunken into the aperture made by the clavicles, and the atrophic arm muscle.

was a syndrome, exclusive of the other signs of the tumor, strongly suggestive of progressive muscular dystrophy.

Finally, Howell's<sup>36</sup> patient developed extreme weakness in one arm and leg and in the muscles of the back so that he showed a tendency to fall backward. His legs gave way when he tried to walk.

34. Hart, C.: Ein Fall v. Angiosarcoma der Glandula pinealis, *Berl. klin. Wchnschr.*, 1909, **16**, 2298.

35. Raymond and Claude: Les tumeurs de la glande pinéale, *Bull. de l'Acad. de méd.*, Paris, 1910, **63**, 265.

36. Howell: Tumors of the Pineal Body, *Proc. Roy. Soc. Med.*, 1910, **3**, 77.

Taking up at this point the manifestations of disease of the pituitary gland, we find it almost banal to refer to the changes in bony structure caused in this way.<sup>37</sup> Especially in hypopituitarism do we see, combined with fatty development and weak musculature, underdeveloped bony tissue<sup>38</sup> with rarefaction. Insufficiency of the suprarenals produces great fatigability in the muscular system;<sup>39</sup> thyroid insufficiency, through its effects on metabolism, produces occasionally changes in joints leading to their immobility and to contractures in the muscles and tendons controlling them.



Fig. 4.—Contractions in the tendons of the arm, the picture giving the extreme limit of extension of the arms and hands possible. Note also the pouting of the lips with a suggestion of the "facies myopathique."

By combinations of these disturbances many of the symptoms exhibited in the cases of progressive muscular dystrophy might be produced. It is highly suggestive that the variations in this most elusive disease, which are enlarged on by almost every investigator, have given rise to almost as many types or forms of the disease as

37. Cushing, H.: *The Pituitary Body and Its Disorders*, 1912. Timme, W.: *Pituitary Disease*, New York Med. Jour., 1915, **102**, 801.

38. Timme, W.: *A Case of Polyglandular Insufficiency with Akromikria*, Boston Med. and Surg. Jour., 1915, **172**, 828; *Conferences of New York Neurological Institute*.

39. Cannon, W.: *Bodily Changes in Pain, Fear, Hunger and Rage*, D. Appleton & Co., 1915.



there are investigators. And this is not to be wondered at if the syndrome depends on as many variables as are represented by the endocrine glands.

The findings at necropsy in the various types of progressive muscular dystrophy do not contradict an internal glandular basis for the disease. Kollarits<sup>40</sup> quotes Blocq and Marinesco,<sup>41</sup> Flandre,<sup>42</sup> and Pennato<sup>43</sup> to show that a relatively negative result, in so far as the nervous system is concerned, was obtained by the more recent investigators. This is to say, in by far the greater number of cases that went to necropsy, no important changes were found in the cerebrospinal system, although in a small number there were minor changes in the anterior horn cells of an atrophic nature. These minor changes are

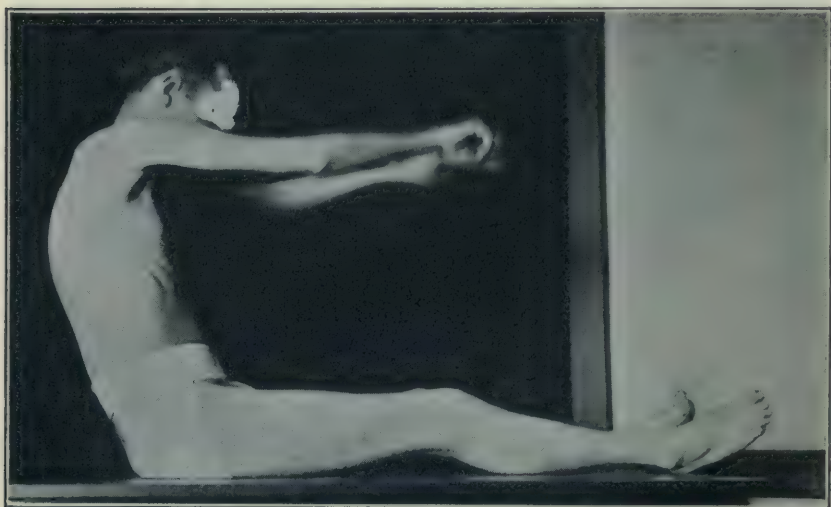


Fig. 5.—Extreme extent of flexion of trunk on thighs that is possible, with legs extended fully, this deficiency being due to the short tendons of the thigh and leg.

not always the same in the different cases and are not proportionate to the clinical disturbances. "For this reason (quoting Kollarits) Erb stated that the dystrophy is probably dependent on functional disturbances of trophic centers not demonstrable anatomically." The actual muscle changes found histologically agree in the main with one another in the different investigations (Spiller,<sup>44</sup> Münzer,<sup>45</sup> Fulda,<sup>46</sup>

40. Kollarits, Jenő: Beitrag zur Kenntniss d. anat. Grundlage d. Muskeltrophie, Budapest Clinic, Division 2, Deutsch. Arch. f. klin. Med., 1901, **70**, 157.

41. Blocq and Marinesco: Arch. d. Neurol., 1893, **25**, 189.

42. Flandre: Thèse de Paris, 1893.

43. Pennato: Clin. med. ital., 1898, **37**, 31.

44. Spiller, W. G.: New York Med. Rec., 1898, **54**, 9.

45. Münzer: Ztschr. f. klin. Med., 1893, **22**, 564.

46. Fulda: Deutsch. Arch. f. klin. Med., 1894-1895, **54**, 525.

Kollarits<sup>40</sup>). The muscles show a large amount of fatty tissue between the muscle fibers, and a few of the muscle fibers with rounded ends. The width of the muscle fiber varies from the normal and the muscle nuclei are increased in number and are grouped together. There is splitting and vacuolization of the muscle fibers and entire disappearance of certain muscles. At times the entire muscle seems to be a gray, fatty mass (Schaedel<sup>47</sup>). The bony changes are at times atrophic in character and at times hypertrophic (Eulenberg<sup>11</sup>).

We can certainly say that postmortem evidence points to a metabolic disturbance. But metabolism in the body is so closely interwoven with the activities of the internal glandular system and the vegetative nervous system that they can hardly be considered apart.



Fig. 6.—Roentgenogram of the forearm and hand, showing the thinness of both ulna and radius and of the phalanges (Patient 24, Fig. 11).

#### AUTHOR'S TYPE OF FAMILIAL PROGRESSIVE MUSCULAR DYSTROPHY

It has been my good fortune to observe several members of a large family during the past year who were affected with a type of progressive muscular dystrophy rather resembling the classic Erb's juvenile form, but differing from it in some particulars. The family tree is herewith given (Fig. 11). The onset and progress of the disease are graphically described by one of those afflicted, a highly intelligent graduate student of one of our foremost universities, and I cannot do better than to give his own description of this insidious affection:

The muscular disease which afflicts our family is similar in all the cases I have investigated. In my own case the trouble appeared at the age of 3, and was noticed by my peculiar method of arising from the floor. The same difficulty was observed in my brother and my sister during early childhood. A general lack of development of the muscles throughout the body marks our disease;

47. Schaedel, Wilhelm: *Zur Lehre v. d. Dystrophia musculorum progressiva*, Dissert., Kiel, 1909.

and there is an accompanying tightening of some of the tendons which limits movement. In the case of my brother the heels are raised from the floor as much as three inches when he stands erect; and my sister is compelled to walk on her toes and the front of her foot so that a special shoe is needed. With the advance of years the difficulty in arising becomes more pronounced. I find it much harder to get up from the floor now than it was seven or eight years ago. My sister, father, uncle and aunt, who have the trouble, cannot arise if they fall down, and they cannot get up from a chair of ordinary height. My sister has put on much weight lately, resembling in this my grandfather, who also had the disease. The latter could not roll over in bed, arise, or walk alone. The

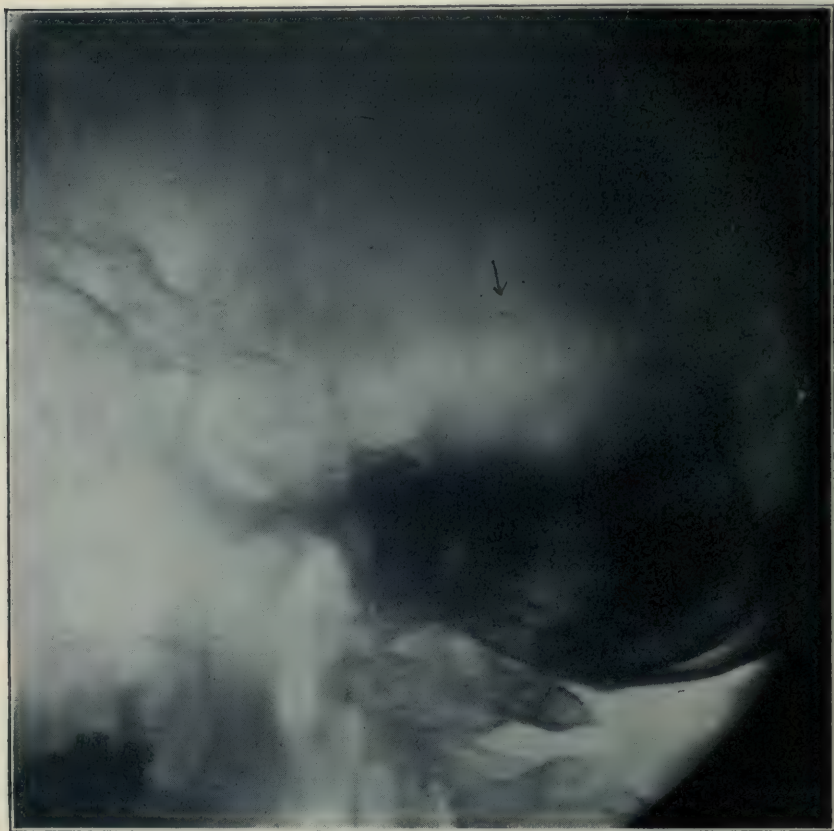


Fig. 7.—Arrow points toward the shadow produced by the pineal gland (Patient 14, Fig. 11).

difficulty in climbing stairs, and in general, in all movement becomes more pronounced with advancing years. The contractions with the drawing-up feature become worse during the growth from childhood to maturity. There is some reason to believe that this contraction continues slowly through the periods of maturity and especially of senescence. The general health of the affected individuals is very good. I cannot remember of illness, or of the report of illness in the case of my father or my uncle; the family is long lived and intellectually sound. There is no bodily pain connected with the disease. There is only a great physical limitation with its accompanying embarrassment. Tradition has it that the original case of the disease arose at the age of 12 following an injury in the field.



Of the fourteen members of the family through three generations who were affected, six have died, at the ages of 88, 73, 71, 69, 64, and 53. Of those living, three are 69, 66, and 64 years old, while the rest are of the present generation in the teens or twenties. That is, the span of life seems rather to be above the average. In this regard, the affection differs from most of those heretofore cited. Gowers,<sup>5</sup> out of a study of 220 cases that he collected, declared that the earlier the disease begins the more severe it is, its usual onset being before 6. Out of thirty of his own patients, twenty-four died between 10 and



Fig. 8.—Roentgenogram of skull of Patient 26 (Fig. 11), a youth 21 years of age, showing shadow in the pineal region.

20; and the oldest lived to be only 40. He also gave the predominant male to female ratio of 190 to 30. In our cases, nine were male and five female. Barsickow,<sup>5</sup> in his study of twenty-four cases, while giving an age at death closely approximating ours, yet declared the onset in all of seventeen known cases to be after the 10th year and mostly between the 20th and 30th years, truly an adult type. So that our cases differ from these two types in the combination of an early onset and a long, rather benign course.

Symptomatology: We have been able either personally or through their own physicians to examine thus far six of the seven living members of the family that have the disease. Patient 24 (Fig. 11) was the one originally examined at the Neurological Institute by us. His examination showed the following:

The patient is a man of 23 years, a student. The station is good, though the attitude is somewhat faulty, as is seen by the photograph (Fig. 1). His head leans forward, with chest retreating; there is some lordosis, and the arms are held at the side partially contracted at the elbow and wrist joints. The gait is peculiar in that he stoops

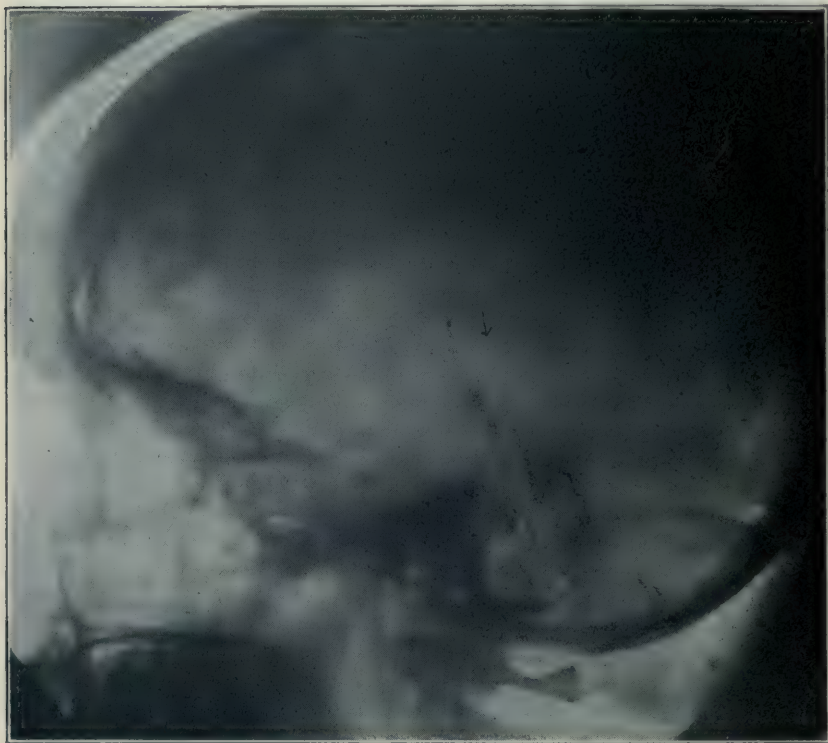


Fig. 9.—Roentgenogram of skull of patient 27 (Fig. 11), a girl 19 years of age. Here the shadow is not so distinct as in the others, but still is present.

his shoulders forwards and toes out. If he doesn't stoop his shoulders, his heels will not touch the floor. There is little or no spring in his walk, his whole body jerking with each foot fall. His weight when he is stripped is 108 pounds.

All the deep and superficial reflexes are normal and equal on both sides; there is no Babinski response and there is no clonus.

There are no sensory changes objectively to be elicited, excepting some pain on pressure over the fourth dorsal vertebra; subjectively

he complains of no pain whatever excepting at times "rheumatic" pains in the joints. This soreness in the joints all the affected members of the family experience. Those unaffected with the dystrophy do not have the joint pains.

There are no signs of involvement of any of the cranial nerves, with exception possibly of those controlling the eye muscles. Every dystrophic member of the family gives a history of eye trouble and

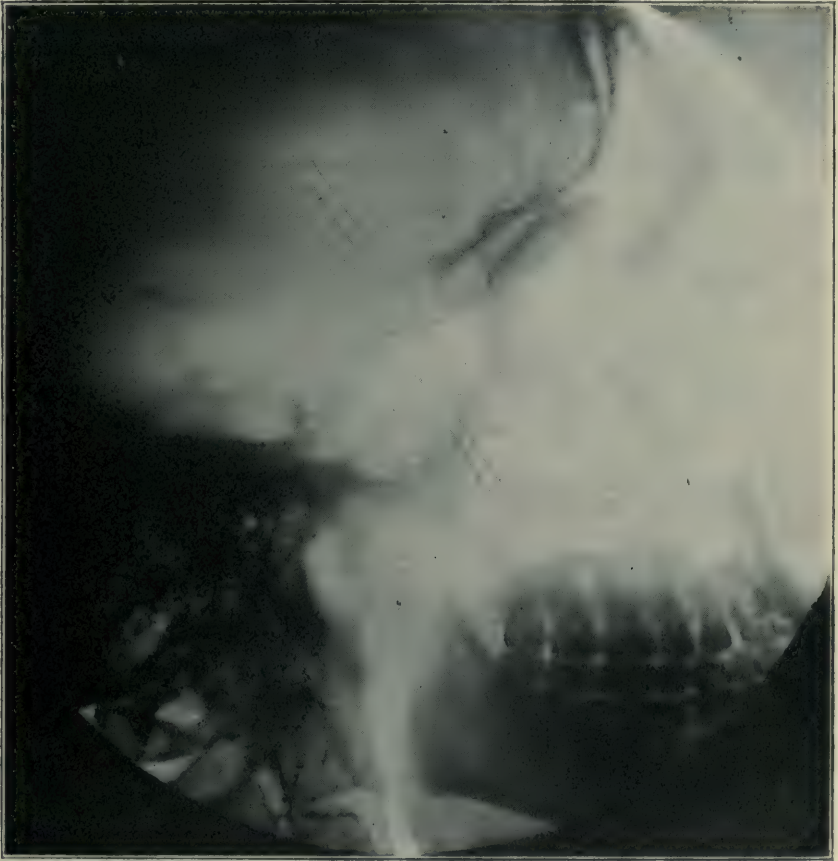


Fig. 10.—Roentgenogram of skull of patient shown in Figures from 1 to 5, inclusive. The shadows in the pineal region are marked (Patient 24, Fig. 11).

must wear glasses. These ocular muscle disturbances are important as referring possibly to the pineal gland in its relation to the anterior corpora quadrigemina. His father, also affected with dystrophy, awoke suddenly one morning, blind, and never entirely recovered his vision. There is no difficulty in swallowing or in speech, and the tongue is protruded in the middle line.



The only external signs of an involvement of the internal glandular system is in the spacing of the incisors, and in the prognathism of the upper jaw. We shall later have occasion to point out the extreme probability of the dependence of the entire syndrome, the bony atrophy, fatty deposits in the muscles, etc., on such a disturbance of the endocrine system.

There is no arteriosclerosis; the pulse rate varies from 68 to 72, increasing on slight muscular effort to 90. The blood pressure, systolic, varies from 125 to 145; diastolic, from 75 to 100.

The patient is highly intelligent, a postgraduate student at an important university, fitting himself for the philosophical degree. His auditory memory is good, while his visual memory is very poor. He is fairly frequently depressed, seclusive and shut-in and has a reaction of embarrassment in company. He feels his disability keenly.

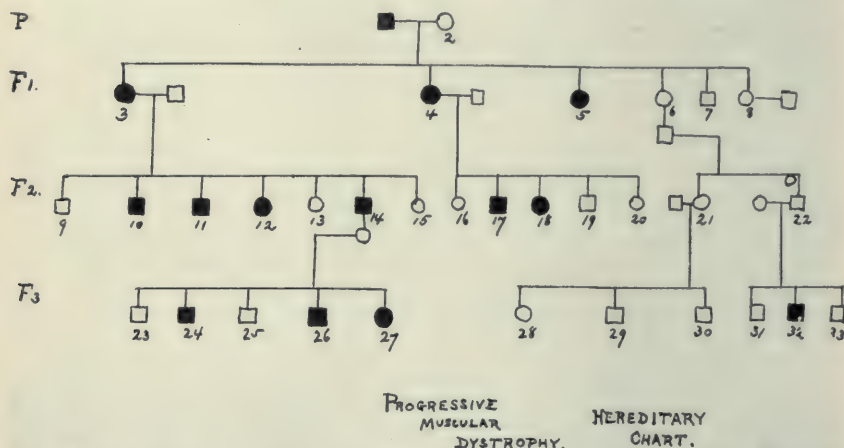


Fig. 11.—Heredity chart of the family in which the dystrophy cases occurred, those in black being the individuals affected.

Urine and blood are quite normal in all particulars. The Wassermann reaction in the serum is negative.

It is in the muscular system that the chief characteristics of the condition lie. The first striking feature is the almost entire absence of the pectorals of both sides, with the exception of the clavicular strands and a few strands of the pectoralis minor (Fig. 3). Both deltoids are very atrophic, and the intrinsic neck muscles are so much so that the neck seems to fit like a funnel into the aperture made by the clavicles and sternum instead of being attached thereto. The trapezius, the serratus magnus and the rhomboids are only partially atrophied. The biceps and triceps are much atrophied; the long supinators less so, while the muscles of the forearm are only slightly affected. In the leg the quadriceps and the adductors are smaller than normal

and quite inefficient, so that walking upstairs or getting up from a squatting position is a task. It is accomplished by climbing up on the thighs by means of the arms (Fig. 2). Of the hypertrophies that are present, the infraspinatus is moderately enlarged, as are the calf muscles. The facial muscles show a slight atrophy, with everted and pouting lips, although there is no difficulty in whistling. The electrical excitability is somewhat increased to faradism in all the muscles, while the galvanic response is unaltered in degree and quality, that is, there is no reaction of degeneration, no myoclonus, or myoidema; there is no asynergy and no incoordination. There are no actual paralyses, but simply weaknesses. The patient can hold his arms out from his body at the level of the shoulder for one and one-half minutes without fatigue. There then ensues a tremor of the entire limb, and he must let the arms fall. The various measurements of the circumference of the limbs are herewith given in millimeters:

		Right	Left
Upper arm.....	{ Upper	230	225
	{ Middle	190	190
	{ Elbow	193	190
Forearm .....	{ Largest	200	202
	{ Smallest	125	127
Thigh .....	{ Upper	465	465
	{ Middle	420	415
	{ Knee	320	330
Lower leg.....	{ Calf	335	325
	{ Thinnest	173	175

There are contractions in the tendons of the arm muscles and of the calf muscles on both sides—contractions, and not contractures. That is to say, by assuming such positions as will give little tension to the muscles of the particular member examined, the patient recognizes no difficulty in fully extending that limb. Thus, if he relaxes the calf muscles by stooping forward in walking, his heels touch the ground; but if he stands fully erect with shoulders thrown back, then his heels do not touch the ground on account of the short Achilles tendons. The photograph showing the arm outstretched to its fullest extent gives an idea of the shortening of the arm flexor tendons, in the resulting flexion of the fingers and wrist (Fig. 4). When the wrist is fully flexed, the fingers can be extended; when the fingers are fully extended and kept in that position, the wrist cannot be extended at the same time. It is these contractions that give the patient his greatest difficulty, for they impede the free use of his limbs and are progressively, although slowly, increasing (Fig. 5).

The Roentgen ray shows that the bones of the arm and forearm are very small and thin, though not shorter than the normal (Fig. 6).

The skull roentgenogram shows a normal sella turcica, but there are several shadows in the region of the pineal gland (Fig. 10). These shadows are three in number, and although discrete, range closely together.

Three other members of this immediate family, Patient 14 (Fig. 11), father of this man, and Patients 26 and 27, brother and sister of the patient, give similar physical signs and symptoms to those exhibited by this patient, with some minor differences. Thus, his sister, 19 years of age, is quite adipose, and has more marked tendon contractions than the others. She constantly walks on her toes as a result of the Achilles tendon shortening (*pes equinus*), and must have special shoes to accommodate this malposition of the foot to her gait. The brother, aged 21, is in many respects the counterpart of the patient, only not quite so far advanced. The father, aged 65, cannot get up from a sitting position without help. There is the same difficulty in all of these members of the family in going upstairs, almost an impossibility to lift themselves from step to step. But all of them are active mentally. The father still attends to his duties as a minister. The one point that they all have in common, and this we wish to emphasize most strongly, is that the roentgenogram in each instance shows shadows in the region of the pineal gland. In the sister's case these shadows are very faint, but still present (Figs. 7, 8 and 9).

One member, No. 32, of a collateral branch of this family, but still descended from the common ancestor No. 1, is affected by a type of the disease which is sufficiently distinct from that of the others to merit individual description. He is a schoolboy of 15, living in a small Pennsylvania town. His difficulty seemingly followed an attack of chickenpox four years ago. He has no difficulty in squatting down or in arising from the floor, thereby differing from every other member with the disease. Within six months of the onset of the disease, his joints became stiff and the seat of calcareous deposits. Various tendon contractions followed, notably the Achilles and the flexors of the fingers. Muscular atrophies, especially of the deltoids, pectorals, trapezius, biceps, and sternocleidomastoids succeeded. The pectorals are very deficient, the sternocleido muscles also, almost like a lead-pencil in thickness. The entire bodily musculature seems to be deficient in volume and gives the appearance of a big bony frame with insufficient covering. The myotatic irritability is increased in the biceps and deltoids. The bony framework is large, the middle finger measuring 105 mm. from the metacarpophalangeal joint to the tip. Both ankles are deformed, with a marked protrusion of the tarsals inward and a *pes planus*. The foot cannot be flexed dorsally on account of a short Achilles tendon, or ventrally on account of a short *tibialis anticus*. This gives the patient a rather queer shuffling gait. There are cal-



careous nodules on many of the phalanges, especially at the terminal joints. On account of atrophic supinators, supination can be carried out only to a very limited extent by the biceps assisting. This syndrome is rather different from that of the other members, in that it began at the comparatively late age of 11 and that it was not accompanied by difficulty in arising or in going upstairs and in its marked disposition to affect the bony skeleton. The Roentgen ray also in this case did not show the pineal gland shadows, but did show a large sella turcica. As the boy is but 15, it is within the realm of probability that within a few years the shadows will appear.

As the members of the various branches of the family are widely scattered, indeed from coast to coast, I have not personally been able as yet to examine all those affected with the disease. The roentgenograms were made by different roentgenologists in various parts of the country, including Dr. Leix of Los Angeles, Dr. Bowen of Philadelphia and Dr. Evans of New York.

To recapitulate, then, we have a somewhat atypical form of progressive muscular dystrophy, rather resembling Erb's infantile type, of extremely benign and slow progress. It occurs as a hereditary affection, now in the fourth generation, which has disabled fourteen individuals. Of the seven living members with the disease, all but two have been examined thus far by Roentgen ray, and of these five, four show distinct changes in the pineal gland producing shadows in the roentgenogram. The fifth, a youth of 15 years, whose affliction is rather of different type than the others, in that the bony growth is abnormal, shows an enlarged sella turcica, but no shadows in the pineal as yet. Weighing all the evidence that is advanced by previous investigators to show derangement of the internal glandular balance in cases of progressive muscular dystrophy, and giving due significance especially to the changes produced by tumors and diseases of the pineal gland on the various tissues of the body, changes that resemble in character, if not entirely in degree or disposition those present in progressive muscular dystrophy, we must admit the extreme probability of a causal relationship between the two. If to this probability we actually adduce the evidence shown by cases of progressive muscular dystrophy of changes in the pineal gland demonstrated by roentgenology, the probability approaches pretty closely to proof that disturbances of the pineal gland play an important rôle in the pathogeny of progressive muscular dystrophy.

In criticism of this deduction, there may be advanced the statement that frequently otherwise normal individuals show shadows in the pineal gland. In answer thereto, Schüller of Vienna, who was the first to demonstrate the identity of the shadows with deposits in the pineal gland, states that in older persons who already have reached

the stage of calcification of the arteries, the shadows are but evidence of such calcified bodies in the pineal gland. But here we have three of the four patients under 24 years of age. The author personally examined about 150 roentgenograms of the skull in the laboratory of Dr. Lewis G. Cole of New York, and found only about 2 per cent. with pineal shadow.

To ask why changes in the ovaries or thyroid or pituitary gland, all of which changes have had their part in the production of symptoms in many of the cases of progressive muscular dystrophy herein cited, should be laid at the door of a diseased pineal gland, brings up the whole question of the interdependence of the endocrine glands. Suffice it to say that the pineal gland is supposed to cease its function at puberty. If earlier or later, then the other glands partially compensate, producing their own particular symptoms in the endeavor to right the wrong. Occasionally this wrong is righted and a cure of the condition produced by the perverted pineal gland results. Thus have come about the several cures reported by Erb, Marina, Levi and Rothschild, and Jendrassik. In each of these cases a rapidly developing gonadal system incidental to puberty produced the cure.

A final report of all the members of this family will be presented by me within the coming year, together with a short disquisition on the therapy of the condition based on the foregoing theory of the pathogenesis of progressive muscular dystrophy, namely, that the disease must be classed as an endocrinopathy.

# BLOOD CHANGES IN ALBINO RATS FOLLOWING REMOVAL OF THE SPLEEN \*

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## INTRODUCTORY

About a year ago, during the course of studies in the albino rat (*Mus norvegicus-albinus*) carried on under the direction of Dr. Alfred Stengel, on the relation of the spleen to the other glands of internal secretion, it was thought worth while to note incidentally any blood changes that might occur after splenectomy, since no references to such observations on the rat could be found in the literature. Among the first observations a few unusual and interesting phenomena were noted, which seemed to make a somewhat systematic study worth while.

A fairly complete bibliography of the literature up to 1914 of the blood changes after splenectomy may be found among the references given by Meyer.<sup>1</sup> The most important work since that time is that of Pearce and his collaborators.<sup>2</sup>

Observations on man, dogs, rabbits, goats, sheep and other animals have shown that removal of the spleen is usually followed by certain alterations in the blood. The time of onset, the extent of the changes and the period of recovery are highly variable. This variability applies not only to the results of different investigators, but also to the data obtained from single series, in which the experimental conditions may be considered fairly comparable. The most important of the blood changes may be summed up as follows:

1. Slight to moderate anemia, variable in time of onset, but usually within the first three or four weeks after splenectomy. Recovery from this anemia is usually complete within a period varying from a few weeks to a few months.

2. Marked rise in leukocyte count followed by a gradual decline. A slight leukocytosis with excess of lymphocytes and eosinophils tends to persist for a long time.

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\* From the William Pepper Laboratory of Clinical Medicine and the Henrietta Hecksher Fellowship in Medical Research, University of Pennsylvania, School of Medicine.

1. Meyer, A.: *Centralbl. f. d. Grenzgeb. d. Med. u. Chir.*, 1914, **18**, 41.

2. Pearce and Collaborators: The Relation of the Spleen to Blood Destruction and Regeneration and to Hemolytic Jaundice, numerous papers, *Jour. Exper. Med.*, 1912-1916.



3. Increased resistance of erythrocytes to hemolytic agents, such as hypotonic salt solution and hemolytic immune serums.

None of the theories offered in the attempt to explain the cause of the anemia of splenectomy have proved adequate. Krumbhaar, Musser and Pearce,<sup>3</sup> as a result of their studies on blood regeneration following bleeding and the administration of hemolytic agents to splenectomized dogs, conclude that the anemia of splenectomy is caused by some factor, as yet unknown, operating in the absence of the spleen.

#### MATERIAL AND METHODS

All litters of rats used in this series were carefully selected from the colony kept at the Wistar Institute of Anatomy and Biology. The animals were kept in the animal house of the Wistar Institute during the course of the work. For these privileges I am indebted to Prof. H. H. Donaldson. I am further indebted to Professor Donaldson for many helpful suggestions during the course of the work.

The ages of the animals at the time of operation varied between 30 and 70 days, averaging about 45 days. In every experiment parallel observations were made on the splenectomized animal and a control of the same sex and from the same litter. Animals operated on and controls were always kept in the same cage. Operations were performed under ether anesthesia. Splenectomy in the rat requires only three to four minutes for its performance. There is practically no loss of blood. Recovery takes place promptly; within an hour the rat will run about the cage and by the next morning is apparently quite as active as the control. No wound infection, peritonitis or evidences of internal hemorrhage were discovered in the series.

The blood for cell counts, hemoglobin estimations and study of reticulated cells was obtained by snipping the end of the tail with a pair of sharp scissors. A fairly free oozing of blood is required to obtain accurate cell counts and hemoglobin estimations. The Fleisch hemometer was used throughout. For the study of reticulated cells brilliant cresyl blue stain was employed, according to the usual technic.

#### BLOOD CHANGES FOLLOWING REMOVAL OF NORMAL SPLEENS

The results of observations on red blood cell count, hemoglobin and reticulated cells are given in Table 1. The results are somewhat variable. Most splenectomized animals show a transient drop in cell count and hemoglobin, reaching its maximum in from one to three weeks. Nearly all appear to have recovered completely by the end of the fourth week after operation. Three out of sixteen show no evidence of anemia, but it is possible that more frequent blood counts

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3. Krumbhaar, E. B.; Musser, J. H., Jr., and Pearce, R. M.: *Jour. Exper. Med.*, 1913, **18**, 665.

TABLE 1.—STUDIES OF RED BLOOD CELL COUNT, PERCENTAGE OF HEMOGLOBIN  
AND PERCENTAGE OF RETICULATED CELLS IN  
RATS WITH NORMAL SPLEENS

Rat No.	Days After Splenectomy	Red Blood Cell Count in Millions		Hemoglobin Percentage		Percentage of Reticulated Red Cells	
		Operated	Control	Operated	Control	Operated	Control
54 and control.....	Preliminary	7.6	7.3	85	78	2.8	7.2
	3	7.1	7.3	80	82	4.6	10.4
	8	5.7	7.2	69	85	2.8	1.0
	16	6.5	8.1	71	82	4.0	8.0
	29	8.2	8.2	80	82	3.4	4.0
	45	7.2	8.7	78	94	8.6	5.2
55 control same as for 54	Preliminary	7.3	....	82	...	3.4	....
	3	7.0	....	81	...	2.4	....
	8	6.0	....	67	...	1.4	....
	16	7.6	....	77	...	16.0	....
	29	8.1	...	84	...	0.6	....
	45	7.4	....	82	...	3.2	....
57 and control.....	Preliminary	9.2	9.2	92	86	2.0	4.2
	14	7.9	8.0	88	87	....	....
	28	7.6	8.3	72	84	1.0	3.2
	44	9.9	8.3	87	84	0.8	1.6
49 and control.....	Preliminary	8.5	9.2	83	91	0.1	0.1
	3	8.2	....	73	...	2.0	2.0
	6	7.7	8.6	79	84	3.4	1.2
	20	8.0	7.6	90	86	0.2	1.8
	34	8.5	7.9	90	84	1.4	1.6
	50	9.4	9.7	96	90	0.2	1.4
	141	10.3	10.7	84	92	0.8	0.6
	254	9.1	8.8	99	104	0.6	0.5
60 and control.....	Preliminary	8.9	8.4	86	90	0.	0.2
	5	9.1	8.8	83	88	1.2	1.2
	13	8.9	8.4	85	87	1.2	2.0
	28	8.0	9.2	84	86	1.4	1.0
	231	9.2	9.4	91	82	1.8	2.2
64 and control same as for 60	Preliminary	8.9	....	90	...	0.2	....
	5	8.4	....	74	...	0.5	....
	13	8.2	....	88	...	3.4	....
	28	8.1	....	82	...	0.4	....
	231	9.7	....	95	...	1.4	....
7 and control.....	Preliminary	8.8	9.0	94	98	....	....
	7	7.6	7.9	76	84	...	....
	14	7.8	9.2	69	86	....	....
	21	7.3	7.8	64	86	....	....
	30	8.7	7.6	88	86	....	....

TABLE 1.—STUDIES OF RED BLOOD CELL COUNT, PERCENTAGE OF HEMOGLOBIN  
AND PERCENTAGE OF RETICULATED CELLS IN RATS WITH  
NORMAL SPLEENS—(Continued)

Rat No.	Days After Splenectomy	Red Blood Cell Count in Millions		Hemoglobin Percentage		Percentage of Reticulated Red Cells	
		Operated	Control	Operated	Control	Operated	Control
10 and control.....	Preliminary	8.5	8.8	94	94	....	....
	6	7.4	7.3	81	88	....	....
	13	7.6	8.2	68	86	....	....
	20	7.7	7.6	75	86	....	....
	29	7.4	8.7	73	88	....	....
19 and control.....	Preliminary	8.6	8.8	96	96	....	....
	7	6.9	7.8	66	82	....	....
	20	6.9	7.5	75	86	....	....
	30	7.2	8.6	70	94	....	....
22 and control.....	Preliminary	9.4	9.7	94	92	....	....
	7	7.4	7.8	78	82	....	....
	20	6.9	7.5	66	80	....	....
	30	6.9	7.5	72	94	....	....
30 and control.....	Preliminary	8.2	....	88	...	....	....
	3	8.5	8.1	78	80	....	....
	13	8.4	8.8	85	95	....	....
	47	9.5	9.9	85	95	....	....
32 and control.....	Preliminary	8.3	....	90	...	....	....
	3	8.4	8.7	90	96	....	....
	13	7.8	8.1	82	90	....	....
	25	8.1	8.2	82	84	....	....
	32	8.9	8.2	84	87	....	....
40 and control.....	Preliminary	7.8	8.0	84	90	....	....
	7	7.3	7.3	84	81	....	....
	14	7.9	7.6	80	79	....	....
	22	7.9	7.9	75	92	....	....
	31	8.2	7.4	88	81	....	....
41 same control as for 40	Preliminary	7.7	....	90	...	....	....
	7	7.9	....	85	...	....	....
	14	7.9	....	86	...	....	....
	22	7.6	....	81	...	....	....
	31	8.9	....	86	...	....	....
73 and control.....	Preliminary	8.1	8.3	85	84	0.8	1.0
	3	7.0	9.8	74	92	3.0	....
	13	7.9	8.1	64	82	2.8	0.8
	25	8.7	8.6	91	90	....	....
	230	9.4	9.3	96	97	1.2	0.8
71 and control.....	Preliminary	7.8	8.6	94	94	1.6	0.8
	3	9.7	9.8	89	90	....	....
	13	7.9	7.7	75	82	2.6	1.4
	25	9.1	8.2	95	98	....	....
	230	8.8	9.1	110	102	3.0	1.0



might have disclosed a slight degree. The drop in red cells is slight, but the hemoglobin percentage shows a distinct change. The average of fifty-eight examinations in the first five weeks after splenectomy is 7,850,000 red cells and 79.2 per cent. hemoglobin; the average of forty-five readings in controls is 8,160,000 red cells and 86.6 per cent. hemoglobin. The average of the lowest readings in sixteen animals operated on is 7,350,000 red cells and 72.9 per cent. hemoglobin, the average of the lowest readings in thirteen controls is 7,740,000 red cells and 82.1 per cent. hemoglobin. The tendency to slightly more marked decrease in hemoglobin than red cells was found in dogs by Musser and Krumbhaar.<sup>4</sup> Five rats examined about eight months after splenectomy showed normal cell counts and hemoglobin.

The leukocyte counts in the albino rat show such variations that conclusions regarding possible changes produced by splenectomy are difficult to draw. There seems to be a tendency toward development of slight leukocytosis.

Examinations of stained specimens of blood from the young rat frequently show a few nucleated red cells. During the period of anemia following splenectomy they are sometimes found in fairly large numbers, as many as five normoblasts to one leukocyte.

The normal variation in percentage of reticulated cells in young rats was found to be large; some showed as high as 16 per cent. In eight adult animals 3 per cent. was the highest number found. No changes that could be attributed to the splenectomy were discovered.

#### RESISTANCE OF ERYTHROCYTES TO HYPOTONIC SALT SOLUTION

Because of the extremely rapid clotting time of rat blood, a slight modification of technic was deemed advisable.

Sodium citrate in 1.5 per cent. solution, just sufficient to prevent coagulation, was added to the blood immediately after its withdrawal. Two small drops of the citrated blood were added to each of seventeen tubes containing graduated concentrations of salt solution, beginning with 0.2 per cent. and increasing by 0.025 per cent. up to 0.6 per cent. The tubes were incubated for two hours, then placed in the ice-box for about sixteen hours, after which the readings were made. The first tinge of red visible throughout the entire solution was considered beginning hemolysis; the first tube showing no distinct red sediment was considered as showing complete hemolysis.

The blood of nine splenectomized animals tested four to five weeks after operation (Table 2) showed in every case markedly greater resistance to hypotonic salt solution than that of controls. Tests made

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4. Musser, J. H., Jr., and Krumbhaar, E. B.: *Jour. Exper. Med.*, 1913, **18**, 487.

about eight months after operation yielded somewhat different results. In one case resistance in animals operated on and control was identical. In four other animals operated on the increase of resistance over that of the control animals was distinctly less marked than that observed in the earlier period. These few studies seem to indicate a tendency to eventual loss of the increased resistance.

TABLE 2.—RESISTANCE OF RED CELLS TO HYPOTONIC SALT SOLUTIONS

Rat No.	Days After Splenectomy	Hemolysis Begins		Hemolysis Complete	
		Operated	Control	Operated	Control
1	30	0.400	.....	0.250	.....
3	..	.....	0.450	.....	0.350
4	30	0.400	.....	0.250	.....
5	..	.....	0.475	.....	0.300
7	30	0.400	.....	0.250	.....
8	..	.....	0.475	.....	0.300
19	29	0.425	.....	0.275	.....
20	..	.....	0.500	.....	0.375
32	34	0.425	.....	0.250	.....
33	..	.....	0.475	.....	0.300
36	34	0.450	.....	0.300	.....
37	..	.....	0.475	.....	0.350
38	35	0.425	.....	0.275	.....
39	..	.....	0.475	.....	0.325
40	35	0.350	.....	0.250	.....
41	35	0.400	.....	0.275	.....
42	..	.....	0.450	.....	0.325
49	254	0.450	.....	0.225	.....
50	..	.....	0.475	.....	0.275
60	231	0.450	.....	0.275	.....
64	231	0.425	.....	0.275	.....
62	..	.....	0.500	.....	0.300
70	230	0.500	.....	0.325	.....
71	230	0.500	.....	0.350	.....
72	..	.....	0.500	.....	0.375
73	..	.....	0.500	.....	0.375

## BLOOD CHANGES FOLLOWING REMOVAL OF ENLARGED SPLEENS

Many investigators have observed the occurrence of enlarged spleen in albino rats. The cause of this enlargement, according to Hatai,<sup>5</sup> has never been determined. Hatai found also that a heavy

5. Hatai, S.: Am. Jour. Anat., 1913, **15**, 87.

liver was likely to be associated with the heavy spleen. Professor Donaldson<sup>6</sup> has observed that otherwise apparently healthy rats kept out in the cold during the winter showed enlarged spleens.

During the course of this investigation eight rats with enlarged spleens were operated on and the spleens removed. These eight rats all came from three litters. The general appearance, nutrition, weight and preliminary blood count in each case were within normal limits and corresponded with the data obtained from the other animals in the same litters. In two litters all the rats were slightly above the average weight for their age. In the third, all, including those with normal as well as those with enlarged spleens, were slightly below the average. No means were found of distinguishing the rat with enlarged spleen from the normal animal until the spleen itself was exposed to view.

The gross appearance of the organ is characteristic. It is large, soft, with somewhat rounded edges. The color is dark, reddish blue. Microscopically there is found engorgement with blood and moderate hyperplasia of the lymphoid and endothelial cells. No evidences of inflammation or excessive hemolysis were observed.

In view of these observations it was thought possible that the enlargement might be of the nature of a functional hypertrophy, either in response to a demand for increased splenic function to compensate for lessened or increased activity on the part of functionally correlated organs or tissues, or as part of a protective mechanism invoked by the body against infection or the products of infection.

The data obtained in seven of these rats by examination of the blood is given in Table 3. In six of the seven the onset of an extremely rapid and severe anemia occurred in from three to five days. In Rat 25 the onset of the anemia was less rapid. It was not observed until the twentieth day after operation. Rat 47 died two days after splenectomy, before any study of the blood had been made. Examination, however, showed intense pallor and distinct icteroid tinge of tissues.

Of the seven rats studied, all showed percentage of hemoglobin under 25 and red blood cell counts under 2,540,000. Six showed red blood cell counts under 2,000,000. These findings were in striking contrast to what was observed in sixteen rats from which presumably normal spleens were removed, in which the lowest hemoglobin estimation was 65 per cent. and the lowest red blood cell count 5,700,000. The leukocyte counts also showed differences. As stated before, while the removal of the normal spleen is followed by a tendency to

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6. Donaldson: Personal communication to the author.



TABLE 3.—STUDIES OF RED AND WHITE CELL COUNTS, HEMOGLOBIN, RETICULATED AND NUCLEATED RED CELLS OF RATS WITH ENLARGED SPLEENS

Rat No.	Days After Splenectomy	Red Blood Cell Count in Millions		Percentage of Hemoglobin		Leukocyte Count in Thousands		Per Cent. Reticulated Red Cells		Nucleated Red Cells	
		Operated	Control	Operated	Control	Operated	Control	Operated	Control	Operated	Control
45 and control...	Preliminary	7.7	7.6	87	83	14.6	13.6	....	...	....*	..
	1	7.8	7.5	91	86	10.8	9.4	....	...	....	..
	2	8.2	7.1	91	88	28.6	20.2	....	...	....	..
	3	7.3	7.6	77	90	23.6	11.2	....	...	....	..
	4	1.8	7.4	24	85	20.4	9.6	....	...	Mod. No.	..
	5	1.9	8.2	25	84	36.8	15.2	....	...	Mod. No.	..
	12	3.9	7.4	53	81	44.6	12.2	....	...	Mod. No.	..
	20	5.0	8.1	77	86	15.4	12.0	....	...	Mod. No.	..
	28	1.8	8.0	29	90	36.2	16.2	65	1.4	Mod. No.	..
	37	7.9	8.3	94	85	34.4	23.0	15	8.0	Mod. No.	..
	43	Died	...	..	..	....	....	....	...	....	..
43 and control, same as for 45	Preliminary	7.6	...	83	..	15.0	....	....	...	....	..
	1	7.3	...	76	..	15.0	....	....	...	....	..
	2	6.8	...	76	..	29.8	....	....	...	....	..
	3	1.9	...	29	..	52.8	....	....	...	3	..
	4	Died	...	..	..	....	....	....	...	....	..
44 control, same as 45	Preliminary	7.4	...	86	..	14.0	....	....	...	....	..
	1	7.6	...	88	..	11.6	....	....	...	....	..
	2	6.6	...	76	..	22.0	....	....	...	....	..
	3	6.2	...	72	..	23.0	....	....	...	....	..
	4	1.6	...	21	..	28.0	....	....	...	....	..
	5	2.2	...	23	..	24.6	....	....	...	....	..
	12	6.2	...	72	..	13.8	....	....	...	....	..
	18	Died	...	..	..	....	....	....	...	....	..
53.....	Preliminary	8.4	...	82	..	16.0	....	0.6	...	....	..
	3	4.7	...	46	..	47.6	....	92	...	Few	..
	4	1.3	...	19	..	....	....	89	...	....	..
	4	Died	...	..	..	....	....	....	...	....	..
52.....	Preliminary	8.5	...	100	..	26.2	....	4.5	...	....	..
	3	2.5	...	26	..	49.6	....	84	...	Mod.	..
	4	Died	...	..	..	....	....	1	....	....	..

TABLE 3.—STUDIES OF RED AND WHITE CELL COUNTS, HEMOGLOBIN, RETICULATED AND NUCLEATED RED CELLS OF RATS WITH ENLARGED SPLEENS—(Continued)

Rat No.	Days After Splenectomy	Red Blood Cell Count in Millions		Percentage of Hemoglobin		Leukocyte Count in Thousands		Per Cent. Reticulated Red Cells		Nucleated Red Cells	
		Operated	Control	Operated	Control	Operated	Control	Operated	Control	Operated	Control
and control...	Preliminary	8.9	7.9	86	88	8.6	12.4	....	...	....	..
	6	6.7	7.3	68	84	15.2	19.0	....	...	....	..
	13	7.8	7.7	77	88	21.4	14.4	....	...	....	..
	20	1.9	8.2	22	83	22.2	10.2	....	...	Many	..
	27	2.0	9.7	38	90	28.4	9.0	....	...	Many	..
	34	2.9	8.2	52	87	34.8	11.6	....	...	Many	..
	41	2.1	8.2	23	88	17.8	17.0	....	...	Many	..
	48	3.5	9.0	60	87	29.8	15.0	....	...	....	..
	55	3.3	8.5	47	92	22.4	12.0	....	...	Mod. No.	..
	62	4.6	8.8	55	90	11.2	15.0	....	...	....	..
	70	3.7	9.6	52	92	11.6	10.8	52.0	3.2	....	..
	71	Died	...	..	..	....	....	....	...	....	..
and control...	Preliminary	8.6	9.5	94	92	10.0	9.8	....	...	....	..
	5	1.9	8.0	24	82	46.4	12.6	....	...	Few	2
	12	2.1	7.4	25	88	12.2	12.4	....	...	Mod.	..
	19	3.3	8.1	52	85	36.2	12.8	....	...	Many	..
	26	1.8	7.7	35	81	33.2	13.2	....	...	Many	..
	33	2.8	7.6	51	89	19.8	12.8	....	...	Few	..
	40	3.6	8.5	49	89	20.8	13.4	....	...	....	..
	47	8.7	8.2	75	89	31.6	10.2	....	...	....	..
	54	3.9	8.5	55	84	19.8	14.0	....	...	Few	..
	61	5.6	8.2	70	88	17.0	18.4	....	...	....	..
	79	6.0	8.0	75	84	10.6	10.4	38.0	1.4	....	..
	78	8.1	9.2	95	102	21.4	10.6	4.3	1.4	....	..
	84	7.3	8.1	85	94	....	11.8	6.2	5.0	....	..
	111	5.6	9.9	74	86	....	....	41.0	4.0	....	..
	125	8.4	8.9	86	88	....	....	3.0	2.8	....	..
	216	9.3	9.2	83	87	20.2	16.1	0.8	5.0	....	..
	Killed	...	...	..	..	....	....	....	...	....	..

\* Where no mention is made of nucleated red cells, it signifies that none were seen during a differential count of 300 leukocytes.

leukocytosis, the changes are not very marked. In this series there is a marked rise in the leukocyte count so that counts of 40,000 to 50,000 are not unusual.

Nucleated red cells were observed frequently, and occasionally in large numbers during the periods of anemia. They tended to disappear as the blood count returned to normal. A few studies of reticulated red cells showed extraordinary rises during the period of anemia. This is shown in striking manner in Rat 53, in which a preliminary count just before splenectomy showed 0.6 per cent. of skeined cells. A count made three days later showed 92 per cent. The following day, only a few hours before death, 89 per cent. of the red cells were reticulated. All observations made on anemic blood showed high percentages of reticulated cells. None of the preliminary counts or those made after the blood had been regenerated gave abnormally high percentages.

Seven of the eight rats succumbed. Four died within four days after operation. Rat 44 had one period of partial regeneration of the blood, but died eighteen days after operation. Rat 25 also had two remissions, in the latter of which the blood count returned completely to normal. This animal died forty-three days after operation. Rat 28, the only animal that recovered, showed three periods of anemia before complete recovery. Examination of the blood 216 days after splenectomy showed it to be normal except for a slight leukocytosis.

In all the five animals examined post mortem, the most striking thing observed was the marked pallor of the tissues and evident extreme anemia. Two showed distinct icteroid coloration of the tissues. No evidences of hemorrhage or infection could be found anywhere in the body. The absence of the lung disease, so prevalent in older rats, was noted. The mortality of 87.5 per cent. in this series of rats with enlarged spleen following splenectomy is in striking contrast to the mortality observed among twenty-five other splenectomized rats, of which one died about four weeks after operation. None of the other twenty-four died during periods of observation ranging from twenty-nine days to four months.

#### COMMENT

It seems probable that there is no important function peculiar to the spleen. The slight transient alterations following splenectomy, together with the new lymphoid tissue, make it seem likely that this lymphoid type of tissue normally shares with the spleen certain of its duties, and in the absence of that organ is capable of assuming a large part of the burden.

If a diseased spleen were removed we should expect the results of splenectomy to be less in degree than usual, because compensation for splenic function had already partially occurred.



Musser has observed that in some chronic conditions the spleen may have been diseased so long and so extensively that a vicarious compensation of its function by other organs may have occurred, thus obscuring the effect of removal. The same idea is expressed by Meyer.<sup>1</sup> The latter would exclude observations after removal of the spleen for leukemia, pseudoleukemia and malaria enlargement, Banti's disease, tuberculosis, echinococcus cyst and purulent affections, from the data of pure experiment, because compensation may be expected to have occurred.

When a normal spleen is removed the alterations which result depend on the capability of related tissues to carry on in entirety the particular functions which had been in part performed by the spleen. The extent of the alterations probably depends in part on the amount and functional capability of the substituting tissues, the duration on the rapidity with which these tissues undergo functional hypertrophy.

If, however, conditions of some sort were present in the body demanding increased function of the type carried on by the spleen, in response to which that organ had hypertrophied, we should not expect to find after splenectomy the large factor of safety which is present in the normal animal. We should expect to find an exaggeration of the phenomena that usually occur after splenectomy.

As far as known with certainty at the present time the only untoward result of splenectomy is anemia. This anemia is variable as to degree and duration, probably depending in an inverse relation on the functional capability of the tissue ready to take the place of the spleen. Therefore, if a truly hyperfunctioning spleen were removed we should expect a severe anemia to develop. Such a result has occurred in all our rats with enlarged spleens.

The cause of the anemia cannot be explained at the present time, but certain phenomena in connection with it stand out prominently and are suggestive in their relation to the rôle of the spleen in the mechanism of blood destruction and regeneration.

The hematogenic function seems not only unimpaired, but capable of tremendous activity in the absence of the enlarged spleen. This is shown during the periods of severe anemia, when at times nearly every cell in the circulating blood is a young form. Thus we are forced to explain the anemia on the ground of increased hemolysis. The rapidity of development of the anemia, the jaundice, the overwhelming preponderance of young red cells, in some cases almost to the exclusion of other types, plainly tell the story of hemolysis.

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7. Musser, J. H., Jr.: *THE ARCHIVES INT. MED.*, 1912, **9**, 592.

## SUMMARY

1. Results of splenectomy were studied in sixteen rats whose spleens were presumably normal; also in eight rats with enlarged spleens.

2. Rats after excision of a normal spleen showed a slight transient anemia, slight tendency to leukocytosis, well-marked increase in resistance of erythrocytes, no change in percentages of reticulated red cells. There was an inconstant increase in the number of nucleated red cells during the periods of anemia.

3. Removal of enlarged spleens was followed by rapid and usually fatal anemia, hyperleukocytosis, marked increase in the number of nucleated and reticulated red cells and, in two cases, by distinct jaundice.

## CONCLUSIONS

1. The variability of results following splenectomy is due to several factors, including the functional activity of the spleen and the functional activity and ability to compensate on the part of the tissues with function similar to that of the spleen.

2. The associated phenomena make it appear almost certain that the anemia which develops after the removal of an enlarged spleen is of hemolytic type; thus more evidence is brought forward that the anemia of splenectomy is of hemolytic origin.

3. The type of function exerted by the spleen in the mechanism of blood destruction and regeneration is necessary to life. Usually after the removal of the spleen there are left in the body other tissues capable of carrying on the function successfully. Under circumstances in which the function cannot be successfully assumed by other tissues, removal of the spleen is attended with disastrous results.

# EXPERIMENTS ON THE ORIGIN AND CONDUCTION OF THE CARDIAC IMPULSE

## VII. SINOVENTRICULAR AND SINO-AURICULAR HEARTBLOCK \*

J. A. E. EYSTER, M.D., AND WALTER J. MEEK, PH.D.

The criterion for sino-auricular heartblock in the mammalian heart that has been hitherto applied, both experimentally in the lower animals and clinically in man, is the occurrence of dropped beats in an otherwise regular auricular rhythm. The only exception to this comprises certain visual observations on hearts dying from asphyxia, made by Hering<sup>1</sup> and others, the significance of which loses its value in the light of subsequent work (Eyster and Meek<sup>2</sup>). Neither the venous pulse nor the electrocardiogram in the higher animals gives evidence of activity in the sino-auricular node distinct from that of the auricle, and attempts to record by suspension methods in the exposed heart contraction of these two regions separately have been rewarded only with inconclusive results. It has seemed to us desirable therefore to carry out experimental procedures that should produce partial and complete sino-auricular block, if such conditions are indeed possible in the mammalian heart, and at the same time record separately the activity of the sino-auricular node and the auricles. This we have done in the present series of experiments by recording the action currents directly from the exposed dog's heart with the string galvanometers.

We have reviewed the experimental and clinical literature of sino-auricular block in two preceding publications.<sup>3</sup> Since the publication of the last paper, four additional clinical cases have been described by Levine.<sup>4</sup> While there are reasons to regard the occurrence of dropped beats in an otherwise regular auricular rhythm as reliable evidence of blocking of the excitation above the auricle, there are, of course, other possible interpretations of this phenomenon and we cannot at present state that we have undoubted proof that sino-auricular block may occur under any condition. The dropped beats may, for example, be due to failure of the pacemaker to generate excitations at certain periods, or to failure of the cardiac tissue to respond,

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1. Hering: Arch. f. d. ges. Physiol., 1900, **82**, 1.

2. Eyster and Meek: Heart, 1914, **5**, 137.

3. Eyster and Meek: Heart, 1912, **4**, 59. Eyster and Evans: THE ARCHIVES INT. MED., 1915, **16**, 832.

4. Levine: THE ARCHIVES INT. MED., 1916, **17**, 153.



for reasons other than failure of conduction. It is the main purpose of this paper to furnish proof of the occurrence of partial and complete sino-auricular block in the mammalian heart.

In preceding papers of this series, especially the one immediately preceding,<sup>5</sup> we have demonstrated that the normal cardiac impulse arising in the upper portion of the sino-auricular node is conducted to the right auricle and to the auriculoventricular node by two separate paths. There is thus sinoventricular conduction to be considered, as well as sino-auricular conduction, and the possibility of blocking in either one or both of these conducting paths.

#### EXPERIMENTAL METHODS

The experimental results to be reported in this paper are derived in part from certain of the experiments yielding data of another nature and reported in the preceding publication and in part from additional experiments. All the experiments were on dogs anesthetized with morphin and ether.

In nine preliminary experiments, the exposed sino-auricular region was subjected to torsion,<sup>6</sup> to gradual clamping, either of the node itself or the node was pulled through a clamp and this tightened down on the surrounding structures, and to the application of nicotin and cocain, in solution, in increasing amounts to the node and contiguous tissues. Electrocardiograms were made from the extremities and comparisons of negativity in different regions of the heart carried out by the direct application of nonpolarizable electrodes to the heart. In none of these was any evidence of partial sino-auricular block obtained and no effort was made to constitute complete block. Torsion and gradual clamping of the node or contiguous tissue produced usually acceleration of the rate, premature beats, and if carried far enough, temporary or permanent removal of the pacemaker to the auriculoventricular node or the coronary sinus portion of this node. Nicotin and cocain produced at first slowing of the discharge of the sino-auricular node, later abolition of its function and removal of the pacemaker. These experiments, therefore, so far as the production of sino-auricular block are concerned, were entirely negative.

We then began a systematic effort to obtain evidence for or against the occurrence of sinus block by a series of experiments carried out as follows: On the exposed heart pairs of electrodes close together (within 1 or 2 mm.) were placed on (1) the sino-auricular node, (2) on the right atrium and (3) on the ventricular portion of the auriculo-

5. Eyster, J. A. E., and Meek, W. J.: *THE ARCHIVES INT. MED.*, 1916, **18**, 775.

6. Erlanger and Blackman: *Am. Jour. Physiol.*, 1907, **19**, 125. These authors have reported the occurrence of dropped beats in artificially perfused rabbits' hearts as a result of this procedure.

ventricular node. Each pair was so arranged that it could be connected to one of two string galvanometers and simultaneous records of the electrical negativity in any two of the above regions made at any time on the same photographic record. Mechanical systoles of the right auricular appendage and apex of the right ventricle were recorded on the same record. In this way we could determine the time interval of negativity between any two of these regions and at the same time determine whether these different regions were manifesting their usual sequence and rhythm. In other words, we have by this method a definite graphic record of the activities of these three regions, and the possibility of determining the relation between the activities of each. After determining the normal relation, gradual and progressive isolation of the sino-auricular node was carried out by

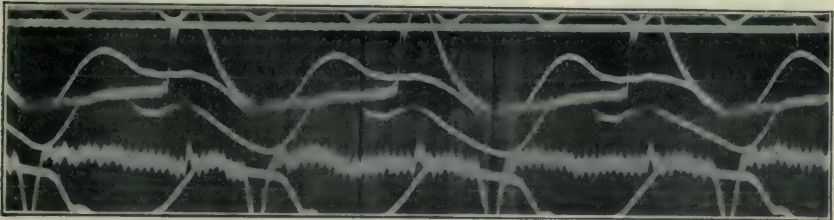


Fig. 1.—Normal beat immediately preceding the occurrence of partial sino-auricular and sinoventricular block in Experiment 34. The record shows, reading from top to bottom, (1) time record in one-fifth second intervals; (2) line made by signal pen; (3) record of mechanical systole of right auricle by lever of recording tambour in front of photographic registration apparatus; (4) shadow of galvanometer fiber connected to two electrodes, one on the upper part, the other on the midregion of the sino-auricular node (upstroke indicates initial negativity of the upper end of the node); (5) duplicate shadow cast by (3), to be ignored in interpretation of record; (6) shadow cast by tambour recording mechanical systole (by downstroke) of right ventricle; (7) galvanometer fiber connected to two points lying about 2 mm. apart on the ventricular portion of the auriculoventricular node.

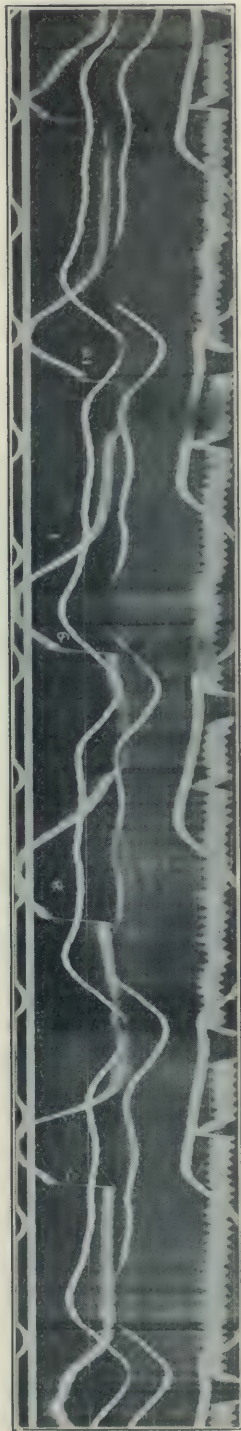
Previous to this record ligatures had been tied across the sulcus terminalis above and below the sino-auricular node and the tissues along the intercaval borders of the node crushed by clamping. As a result of these procedures the *Sa-Av* conduction time had increased from a normal of 0.022 of a second to its average value of 0.03 of a second, as shown in the record from which the figure is reproduced. The average *As-Vs* is 0.18 of a second; the cycle length, 0.50 of a second.

cutting through the tissues around the node, by clamping and, in a few cases, by ligature. The methods employed for the cuts and for clamping are described in detail in the preceding paper.<sup>5</sup> Usually we attempted to make as much permanent separation of tissue by cuts as could be done without causing removal of the seat of impulse formation from the sino-auricular node and then to subject the remainder, or part of the remainder, to gradual clamping, during which periods continuous records were made. Most of our attention was directed





Part 1



Part 2

Fig. 2.—The establishment of partial 5 to 4 and 4 to 3 sino-auricular and sinoventricular block as a result of compressing the tissues along the auricular borders of the sino-auricular node after ligation above and below and after crushing the tissues along the intercaval border of the node. The record is from the same experiment as the preceding and immediately follows that of Figure 1. It is to be interpreted similarly. The first cycle of the record shows a blocked sino-auricular impulse. The second cycle shows an approximately normal *Sa-Av* conduction period of 0.04 of a second. In the third cycle *Sa-Av* conduction time has increased to 0.17 of a second, in the fourth to 0.30 of a second, and in the fifth to 0.45 of a second. The sixth impulse arising in the sino-auricular node fails to be conducted. The seventh sino-auricular impulse shows again a shortening of the *Sa-Av* interval to 0.08 and then follows a progressive lengthening in the eighth and ninth cycles until the tenth sino-auricular impulse fails to be conducted. Finally, the eleventh sino-auricular impulse, following the blocked cycle, shows an *Sa-Av* conduction period of 0.023 of a second.



to the normal sinoventricular conduction path, the path between the sino-auricular and auriculoventricular nodes, and the records taken during the above procedure comprised the movements of the galvanometer connected with these two regions along with the records of mechanical systoles. In some of the experiments, finally, suspension records of the right auricle and ventricle were made on smoked paper, in addition to the photographic records.

INCREASE OF SINOVENTRICULAR AND SINO-AURICULAR CONDUCTION  
TIME AS A RESULT OF PARTIAL ISOLATION OF THE  
SINO-AURICULAR NODE

As has been reported in the preceding paper of this series, there is usually a definite increase in the time of conduction from the sino-auricular node to the auriculoventricular node as a result of partial interruption of the paths between these two regions. The normal period may increase from 0.02 or 0.03 of a second to 0.05 of a second. An interesting fact was not infrequently noted in this connection, namely, that the increase in conduction time was usually greater immediately after the interruption than later, a partial return to the normal conduction time usually occurring after a few seconds. Reference may be made to Table 6 of the preceding paper for these data. The time of conduction between the sino-auricular node and the right auricle, the sino-auricular period, was usually unaffected by partial isolation of the node, except along the auricular border. This latter procedure resulted in a definite increase. Finally, it is to be noted that in a number of instances it was possible to have, after the complete abolition of the normal sinoventricular conduction path, the conduction of the excitation from the sino-auricular node to the auriculoventricular by way of the right auricle. Under these circumstances conduction time between the sino-auricular and auriculoventricular nodes was usually considerably prolonged, while sino-auricular conduction time was of normal length or in some cases somewhat diminished. This occurred in only 27 per cent. of all cases; in 73 per cent. conduction to the auriculoventricular node by way of the right auricle was apparently so difficult that auriculoventricular rhythm was at once established when the normal sinoventricular conduction was abolished.

Depression of conductivity in the normal path of conduction between the sino-auricular and auriculoventricular nodes (sinoventricular path) leads therefore at first to increase in this conduction time. Further depression may lead to conduction by an abnormal path, that by way of the right auricle, in about one fifth of all cases, or it may lead to complete blocking of the impulse and the establishment of auriculoventricular rhythm.



node—auriculoventricular node (*Sa-Av*) conduction period from a normal of 0.022 to 0.05 of a second, then the occurrence of auriculoventricular rhythm, and finally the return of sinoventricular rhythm. Later this conduction period was gradually reduced to 0.03 of a second. At this stage of the experiment a clamp was applied along the auricular border of the node and gradually tightened. There was an open space of several millimeters between the clamped area along the auricular border of the node and the ligature that interrupted the path below the node. Gradual tightening of the clamp established the condition shown in the record of Figure 2. This rhythm is clearly that of partial sino-auricular and sinoventricular heart block, in which the sino-auricular node is generating five or six impulses during a period in which only four or five of these are conducted to the auricle. There is a gradual progressive increase of *Sa-Av* conduction until failure of conduction occurs, and one sino-auricular impulse is blocked. Following this, sino-auricular and sinoventricular conduction attains its shortest value, progressively to increase until blocking again occurs. The results from a similar record showing 5 to 4 and 4 to 3 partial block are diagrammed in Figure 3. Screwing down the clamp as tight as possible and interruption of the path in the open space between the clamp and the ligature below the node, by means of another clamp, stopped all conduction from the sino-auricular node and produced auriculoventricular rhythm. The degree of partial sinus block was not increased by this procedure, even temporarily; the passage to auriculoventricular rhythm was abrupt.

So far as we have been able to find, this is the first time that absolutely definite proof has been obtained for the possibility of partial sinus block in the mammalian heart. With the exception of certain suspension curves of very doubtful interpretation supposed to show graphically the contraction of the sino-auricular region,<sup>7</sup> the previous criterion that has been applied to the occurrence of sinus block, both experimentally and clinically, is the dropping of beats in an otherwise regular auricular rhythm or the sudden doubling or halving of the auricular rate. It is noteworthy in this connection that in the one instance of partial sinus block that we have obtained in this series of experiments the suspension curves give absolutely no indication of the condition or indeed suggest any abnormality in rhythm. The true condition is only evident from the relation of the activity of the sino-auricular and auriculoventricular nodes, as registered by the action currents produced in these regions.

Our experiments would seem to establish, in addition to the possibility of sinus block in the mammalian heart, the conclusion that such

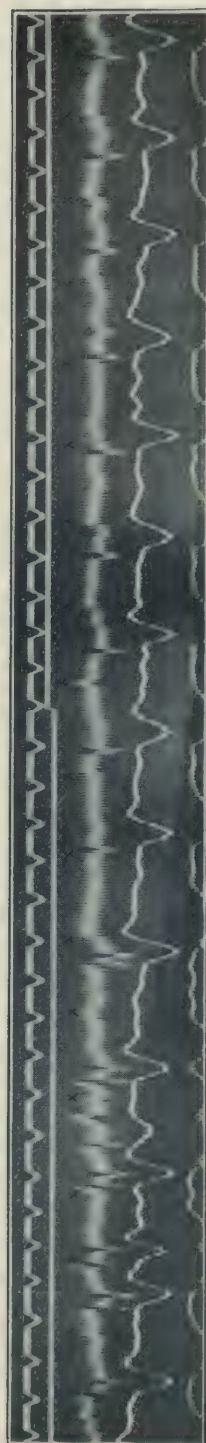
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7. Rehfish: Arch. f. Anat. u. Physiol., 1906, Supplement, 152.





Part 1



Part 2

Fig. 4.—Complete sino-auricular and sinoventricular heart block, produced by clamping along the intercaval border of the sino-auricular node after ligatures had been tied above and below and a cut had been made along the auricular border. The record is similar to that of Figures 1 and 2, except that only one galvanometer was employed, connected between the upper end of the sino-auricular node and the right auricle. At the beginning of the record the electrical complexes associated with each heart beat show waves preceding auricular and ventricular systoles and in addition a small wave (marked *x*) which is the first event in each cycle. As a result of tightening the clamp along the intercaval border the time interval between this wave and the auricular waves at first increases and at the fifteenth or sixteenth cycle after the beginning of the clamping period (as recorded by the signal pen) complete dissociation is evident. Simultaneous systole of the auricles and ventricles is not evident until several cycles later. This condition continues to the end of the record. The small waves, indicative of activity in the sino-auricular node, continue at a more rapid rate and independent of the rate of the auricles and ventricles.

a condition is established only with difficulty. The difficulty of establishment and comparative rarity of partial sino-auricular and sino-ventricular heart block in the mammalian heart is particularly striking in comparison with the ease of establishment and frequency of auriculoventricular heart block. While there may be some factors in this connection with which we are unfamiliar or only partially aware of, we believe that our work has given the possibility of a reasonable answer to the question as to the cause of this wide difference in the frequency of establishment of partial sinus block and partial auriculoventricular block. The reason that sinus block is so rarely met with and so difficult to reproduce experimentally we believe to be due to three main factors: (1) the diffuse nature of the path of conduction from the sino-auricular to the auriculoventricular node; (2) the possibility, in a certain percentage of cases, that when the normal sino-ventricular conduction path is abolished, conduction between the two nodes may occur by way of the right auricle; (3) the usual relatively small difference between the automaticity of the sino-auricular node and the auriculoventricular node. The nature and position of the normal path of conduction between the sino-auricular and auriculoventricular nodes, the normal conduction of the excitation, arising in the sino-auricular node to the right auricle and to the auriculoventricular node by two separate paths with the possibility of conduction by one path when the other is abolished, and finally the relative automaticity of the two nodes have been discussed in detail in the preceding paper on this series. The diffuse nature of the normal sinoven-tricular path necessitates a widespread lesion or other influence depressing normal conductivity, in order to interfere with conduction to the point of serious difficulty or abolition. This is in marked contrast to that present in the conductive system between the auriculoventricular node and the ventricles, which occurs for a part of its distance over a very restricted path, the auriculoventricular bundle. The most important factor tending to prevent the occurrence of partial sinoven-tricular block is, however, the relatively high automaticity of the auriculoventricular node in the mammalian heart. In thirty-four experiments, reported in the preceding paper of this series, in which auriculoventricular rhythm developed as a result of eliminating sino-ventricular conduction, the auriculoventricular node discharged at a rate equal to an average of 67 per cent. of the original sino-auricular discharge. In other words, in the average dog's heart the auriculoventricular node may be regarded as possessing an inherent automaticity equal to 67 per cent. of the automaticity of the sino-auricular



node.<sup>8</sup> It is evident that if the condition of depressed conductivity between the sino-auricular and auriculoventricular nodes developed to the point at which every other or even every third impulse arising in the former node would be blocked, the automaticity of the auriculoventricular node would at once become dominant and auriculoventricular rhythm appear. In the average dog's heart, therefore, the relations of automaticity between the two nodes are such that a partial sino-auricular block of greater degree than that represented by the blocking of every third or fourth impulse from the sino-auricular node would be impossible. Two to one partial block would be possible only in these cases in which the automaticity of the auriculoventricular node was less than half that of the sino-auricular node. This condition was not met with in the thirty-five experiments mentioned above nor in any of the experiments of the present series.

It seems evident, therefore, in order for sino-auricular block of a type approaching that seen in auriculoventricular block, a 2 to 1 or even a 3 to 2 block, to develop, at least two conditions must be present: (1) a degree of depression in the sinoventricular conducting path of exactly the right amount to block every other or every third sino-auricular impulse and no more; and (2) a greater difference in automaticity between the sino-auricular and auriculoventricular node than the average or indeed that which seems normally to exist. It seems probable that in any case in addition to the depression in conductivity there must be a depression also of the normal automaticity of the auriculoventricular node.

The previously reported cases of supposed clinical and experimental sino-auricular heart block, we believe, show certain interesting features in this connection. Of the nine clinical cases in which the absence of beats in an otherwise regular auricular rhythm were satisfactorily determined<sup>9</sup> in only one was the condition ever such as to lead to the interpretation of a condition of a partial sino-auricular block in which more than every other impulse was blocked (2 to 1 sino-auricular block). In this case (Case 2 of Levine) pauses equal to as long as five normal heart cycles were encountered. It is significant in this case, however, that at times, as a result of prolonged blocking of the sinus impulses, the heart began beating, not in auriculoventricular rhythm, but with an independent ventricular (idioventricular) rhythm. It is fair to conclude, therefore, in this case that the automaticity of the auriculoventricular node was much depressed or

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8. Certain experiments which we have performed on the cat's heart, and which will be reported subsequently, indicate an even closer relation between the automaticity of the sino-auricular and auriculoventricular node (particularly the auricular portion or coronary sinus region) in these animals than in the dog.

9. Eyster and Evans: Footnote 3, second reference. Levine: Footnote 4.



abolished. The usual type of sino-auricular block that has been reported clinically in man consists of occasional dropped beats. In the case reported by Eyster and Evans,<sup>10</sup> 2 to 1 block was observed occasionally, never a greater degree. Auriculoventricular rhythm developed at times. The rate of discharge in this latter rhythm was evidently such as to prevent the development of a greater degree of partial block. In at least five of the clinical cases that have been reported the condition of block developed only after the administration of digitalis. The presumption in these cases is that the block developed as a result of depression of conductivity induced by this drug. In the case of Eyster and Evans, in which block occurred independently of digitalis administration, there was abundant evidence that the condition was associated with a hypervagotonus.

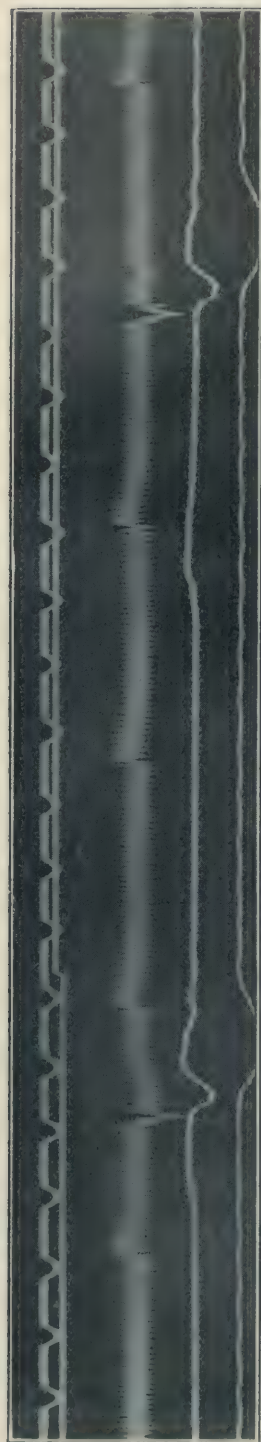
Of the previously reported probable instances of experimental sino-auricular block, that of Cushny<sup>11</sup> following the administration of aconitin and that of Eyster and Meek<sup>8</sup> resulting from large doses of morphin were undoubtedly the result of the action of these substances on the vagal mechanism, and one can readily assume, along with the depression of conductivity, a depression of automaticity of the lower automatic regions. Possibly these substances produce block by a certain degree of selective action on these parts. There is no evidence that stimulation of the vagus mechanism by the usual experimental methods ever produces this result. In Cushny's experiments the evidence pointed to 2 to 1 partial block. In the experiments with morphin the usual type was 2 to 1, but a rhythm interpreted as 3 to 1 block occurred at times. Independent ventricular beats of idioventricular type occurred after prolonged blocking of the sinus impulse, a further indication of the depression of the normal degree of automaticity of the auriculoventricular node.

We feel that it is clear, therefore, that two conditions must be fulfilled in order for a degree of sinoventricular or sino-auricular block greater than the occasional blocking of an impulse to develop in the mammalian heart: (1) a widespread disturbance in conductivity in the regions surrounding the sino-auricular node, and (2) a depression of the inherent automaticity of the auriculoventricular node below its usual value. The infrequency of the fulfilment of those two conditions, we believe, explains the comparative rarity of this condition clinically and the difficulty of its attainment experimentally. The conditions are in marked contrast to those present in the auriculoventricular conductive system. Here the conductive path in a part of its course is markedly circumscribed, and great differences exist between the automaticity of the sino-auricular node or even the auriculoventricular

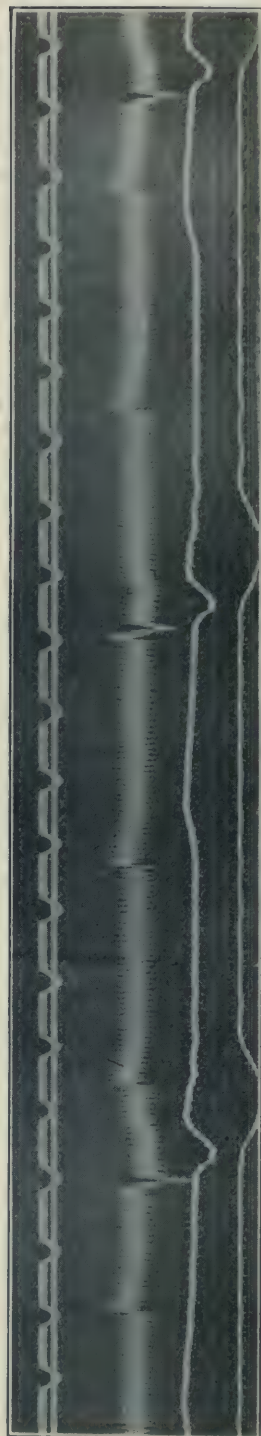
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10. Eyster and Evans: Footnote 3, second reference.

11. Cushny: *Heart*, 1910, **1**, 1.



Part 1



Part 2

Fig. 5.—Influence of stimulation of the vagus on complete sino-auricular and sinoventricular heart block. The record is similar to that of Figure 4 and is from the same experiment. Complete block was induced by the clamp along the intercaval border. The record shows the end of a period of vagal stimulation. The chronotropic effect is much greater on the auriculoventricular than on the sino-auricular rhythm.

node and the automatic or idioventricular centers. Exact figures for the rate of discharge in idioventricular rhythm in the dog's heart compared with the normal sino-auricular rhythm are unavailable, but it is certainly less than half. The ventricle in complete auriculoventricular heart block in man rarely beats at a rate greater than 30 per minute and frequently as low as 15 to 20 beats per minute, or from one-third to one-fourth the normal sinus rate.

Experiments reported previously by us<sup>12</sup> have led us to the conclusion that while normally the excitation spreads from its origin in the sino-auricular node directly to the right auricle and by a separate and diffuse path to the auriculoventricular node, two other possible paths may be employed. Interruption of the normal sinoventricular path leads to the spread of the excitation from the sino-auricular node to the auriculoventricular node by way of the right auricle in about 27 per cent. of all cases. In these instances the sino-auricular and sinoventricular paths are in part common paths. In the remainder auriculoventricular rhythm develops. Interruption of the normal sino-auricular path does not abolish the reception of excitations by the auricle. Under those circumstances, if the sinoventricular conduction path is intact, it receives its excitations apparently by way of some part of this path, or perhaps from some part of the auriculoventricular node. According to this interpretation, partial sino-auricular block is impossible when the natural sinoventricular path is intact, and for this we have abundant experimental evidence. In a number of experiments gradual clamping of the direct auricular connections of the sino-auricular node has been carried out without evidence of block. Complete interruption of these connections leads to an increase in sino-auricular conduction time, evidence of the more circuitous route taken by the impulse, but each impulse arising from the sino-auricular node reaches the auricle. Likewise, sinoventricular block, partial or complete, as a result of interruption of the normal sinoventricular path alone, is impossible in those cases (about 27 per cent.) in which the possibility of conduction to the auriculoventricular node by way of the right auricle is present. In the remainder partial sinoventricular block, independent of sino-auricular block, is conceivable and should be obtained experimentally if a sufficient number of careful attempts are made.

#### COMPLETE SINO-AURICULAR BLOCK

Interference with the conduction in the sinoventricular path to the point of its abolition leads to conduction to the auriculoventricular node by way of the right auricle or, as occurs more frequently, the assumption of dominance in automaticity by the auriculoventricular

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12. Eyster, J. A. E., and Meek, W. J.: Footnote 5; *Heart*, 1914, **5**, 119.



node and the occurrence of auriculoventricular rhythm. In these cases in which conduction to the auriculoventricular node persists by way of the right auricle, auriculoventricular rhythm is in all cases produced finally by interruption of this conduction path. Every case of auriculoventricular rhythm produced by abolition of the sinoventricular conduction paths may be considered as potentially one of complete sinoventricular heart block. Whether this condition can be actually constituted depends on whether one can prove that the sino-auricular node continues in activity under those circumstances. This we have succeeded in doing in two of the cases of auriculoventricular rhythm occurring in the experiments reported in this paper (Experiments 20 and 25). In the remainder there has been no evidence obtainable that the sino-auricular node continues to discharge when its function as pacemaker for the heart has been taken over by a region of lower

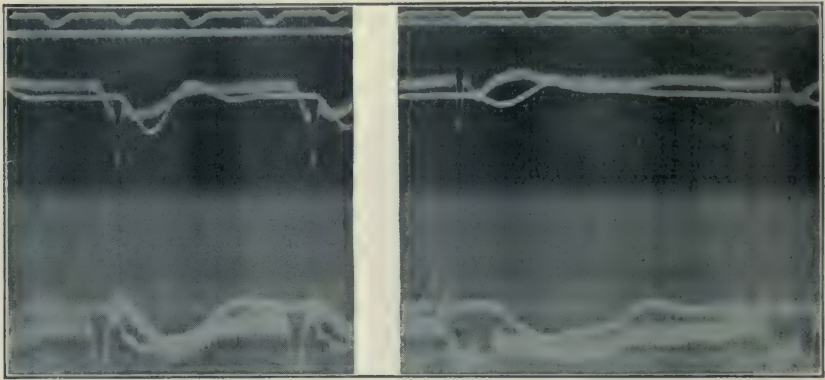


Fig. 6.—Vagal stimulation during auriculoventricular rhythm may lengthen the *As-Vs* interval without causing a return of the pacemaker to the sino-auricular node. The records are to be interpreted as Figures 1 and 2. The left part of the illustration shows the condition before, and the right, during stimulation of the right vagus nerve.

automaticity. These two instances demonstrate for the first time, however, the experimental possibility of complete sinoventricular block, that condition in which the sino-auricular node continues to generate impulses at approximately its normal rate and at a faster rate than the other region which is acting as the seat of origin of the heart beat. The excitations, arising within the sino-auricular node, exert no influence because of the interruption of the conduction paths connecting this region with the other parts of the heart (Figs. 4 and 5).

When the normal path of conduction from the sino-auricular node to the right auricle is abolished, the right auricle may continue to receive impulses from the node by way of the sinoventricular path, as described above. Interruption of the direct auricular connections

of the right auricle with the sino-auricular node results, therefore, not in sino-auricular block, as might be expected, but with merely a delay in conduction time from the node to the auricle.

Theoretically, one might obtain partial or complete sinoventricular block with normal sino-auricular conduction. In these cases in which conduction of the excitation from the sino-auricular node to the auriculoventricular node by way of the right auricle is impossible, and in which, therefore, interruption of the normal sinoventricular path results in auriculoventricular rhythm, it is conceivable that the right auricle might continue to receive its excitations from the sino-auricular node and thus beat at a faster rate than the remainder of the heart. This condition we have never met with, however, in any of our experiments.

#### SUMMARY

1. The possibility of partial and complete sinoventricular heart block has been constituted in the mammalian heart by independent registration of the activities of the sino-auricular node, right auricle and auriculoventricular node by means of the action currents produced in these regions.

2. Since normally conduction from the sino-auricular node to the right auricle and to the auriculoventricular node and ventricular conductive system occurs by two separate paths, a sino-auricular path and sinoventricular path, possibilities of block in either path must be considered. In a certain percentage of experiments it has been found that abolition of the sinoventricular path results in utilization of the sino-auricular path for conduction to the auriculoventricular node. The single case of partial block described in this paper was concerned with depression of conduction in this path and was therefore combined sinoventricular and sino-auricular block.

3. It is believed that there are two important factors which tend to prevent the establishment of partial sinoventricular block and which render it so difficult to obtain experimentally and explain its extreme rarity as a clinical condition. These are the diffuse nature of the path concerned in sinoventricular conduction and the relatively high automaticity of the auriculoventricular node. This latter region is probably always more than half as automatic as the sino-auricular node. The establishment of partial sinoventricular block of a grade equal to or greater than that of a 2 to 1 relation requires, therefore, the simultaneous operation of two factors, a depression of conductivity in the sinoventricular path of a certain definite degree, and a depression of the normal automaticity of the auriculoventricular node without any depression or with a less degree of depression of the sino-auricular node.



4. Complete sinoventricular block is a possible condition whenever auriculoventricular rhythm develops as the result of abolition of the sinoventricular conduction path. If the sino-auricular node continues under these conditions at its normal rate or at a faster rate than the remainder of the heart, the condition is one of complete sinoventricular block. We have been able to obtain this condition in two of the cases of auriculoventricular rhythm resulting in those experiments from abolition of the sinoventricular conduction path.

#### SUMMARIES OF EXPERIMENTS

EXPERIMENT 1.—Electrocardiograms were made throughout the experimental procedures from Lead II. Torsion on exposed sino-auricular node by means of fine-pointed forceps produced shortening of *P R* interval. The gradual clamping of nodal tissue produced fusion of the *P* and *R* waves. There was no evidence of partial block at any time.

EXPERIMENT 2.—Electrocardiograms were made from Lead II. Suspension curves of right auricle and right ventricle. The upper portion of sino-auricular node was caught in blunt forceps and submitted to torsion and twisting. There were numerous extrasystoles, but no evidence of block. Clamping the node produced coronary sinus rhythm, as indicated by the shortening of the *As-Vs* interval, lengthening of the cycle, and so confirmed by the determination of the point of initial negativity.

EXPERIMENT 3.—Electrocardiograms were made from Lead II. Comparisons were made to determine the seat of initial negativity and the relation of negativity in different regions to mechanical systole of the right auricle. Suspension curves of right auricle and right ventricle. The gradual clamping of the sino-auricular node with a specially devised clamp produced auriculoventricular rhythm with *As-Vs=O*; *P* and *R* fused. There was recovery of the normal rhythm on loosening the clamp. There was no evidence of partial block on passage into or recovery from auriculoventricular rhythm.

EXPERIMENT 4.—This was similar in every way to the last experiment. The clamping produced coronary sinus rhythm. There was no evidence of block.

EXPERIMENT 5.—This was similar to the last experiment. Clamping of the sino-auricular tissue produced numerous extrasystoles, an attack of auricular flutter, and finally recovery with heart in coronary sinus rhythm. There was no evidence of block.

EXPERIMENT 6.—This experiment was similar to the last, except that 10 per cent. cocain solution was applied to the sino-auricular node. This resulted in passage into auriculoventricular rhythm without evidence of block.

EXPERIMENT 7.—This was similar to the last experiment. Several applications of 10 per cent. and 20 per cent. cocain solution caused at first temporary slowing of the sino-auricular rhythm without a change in the seat of the pacemaker. Later, a period of auriculoventricular rhythm resulted with subsequent recovery of normal sino-auricular rhythm. There was no evidence of partial block at any time.

EXPERIMENT 8.—This was similar to the last experiment. Various strengths of nicotin applied to the sino-auricular node, namely, 1, 5, and 10 per cent., produced slight slowing of the sino-auricular rhythm without change in the seat of the pacemaker. Twenty-five per cent. caused transitory auriculoventricular rhythm with each application. There was no evidence of partial block.

EXPERIMENT 9.—This was similar to the last experiment. Weaker strengths of nicotin produced slowing of the sino-auricular rhythm. Transitory periods of coronary sinus rhythm were caused by 25 per cent. nicotin. There was no evidence of block at any time.



In most of the following experiments two galvanometers were employed with measurements of relative time of onset of negativity in different regions and changes in these relations during and following partial or complete isolation of the sino-auricular node.

EXPERIMENT 10.—Normally  $Sa-Ra=0.016$ ,  $Sa-Av=0.028$ ,  $As-Vs=0.095$ , cycle=0.38. Tying a ligature across the sulcus terminalis below the node produced no change with the exception of several extrasystoles during the act of tying. Tying the ligature along the venous border of the sulcus produced an auriculoventricular rhythm lasting for about ten minutes, with  $Av-Sa=0.015$ ,  $As-Vs=0.03$ , cycle=0.44. Sino-auricular rhythm returned with  $Sa-Av=0.04$ ,  $Sa-Ra=0.02$ ,  $As-Vs=0.07$ , cycle, 0.41. There was no evidence of partial block during the tying of the ligatures or during the period of recovery of sino-auricular rhythm.

EXPERIMENT 11.—Normally  $Sa-Ra=0.03$ ,  $Sa-Av=0.012$ ,  $As-Vs=0.08$ , cycle=0.33. A ligature was tied across the sulcus below the node. This produced transitory coronary sinus rhythm ( $Sa-Av=0.005$ ), with return of sino-auricular rhythm within one minute.  $Sa-Av=0.023$ ,  $As-Vs=0.09$ , cycle=0.35. Tying a ligature along approximately the lower half of the intercaval border of the sulcus produced coronary sinus rhythm, with  $Sa-Av=0.005$ ,  $As-Vs=0.06$ , cycle=0.38. This rhythm was confirmed by direct comparisons of onset of negativity. Transition to this rhythm occurs within one beat without evidence of block. Coronary sinus rhythm persisted for about thirty minutes, when sino-auricular rhythm returned with  $Sa-Av=0.035$ ,  $As-Vs=0.08$ , cycle=0.38.

EXPERIMENT 12.—Normally  $Sa-Ra=0.011$ ,  $Sa-Av=0.026$ ,  $As-Vs=0.11$ , cycle=0.33. A ligature was tied along the intercaval border approximately the lower half of the sulcus. This caused a shift of the pacemaker from the upper to the lower end of the sino-auricular node.  $Sa-Av=0.008$ ,  $As-Vs=0.08$ , cycle=0.42. A ligature was tied across the sulcus below the sino-auricular node. This produced auriculoventricular rhythm:  $Av-Sa=0.025$ ,  $As-Vs=0.04$ , cycle=0.47. The record taken during the tying of this ligature shows no evidence of partial block. Later  $Av-Sa=0.025$ ,  $As-Vs=0.05$ , cycle=0.48.

EXPERIMENT 13.—Normally  $Sa-Av=0.028$ ,  $As-Vs=0.12$ , cycle=0.27. Two ligatures along the intercaval borders of the sulcus, together extending the full length of the node, produced no effect. Ligation across the sulcus below the node lengthened  $Sa-Av$  to 0.034.  $As-Vs$  and cycle remained unchanged. Ligation across the sulcus above the node produced auriculoventricular rhythm of the coronary sinus type.  $Av-Sa=0.003$ ,  $As-Vs=0.08$ , cycle=0.33. The record taken during the development of this rhythm shows no evidence of partial block.

EXPERIMENT 14.—Normally  $Sa-Av=0.026$ ,  $Sa-Ra=0.02$ ,  $As-Vs=0.10$ , cycle=0.43. A cut was made along the auricular border of the sulcus. There was no change except  $Sa-Ra$  lengthens to 0.026. A cut made across the sulcus below the node produced no further change. A cut along the intercaval borders of the sulcus produced auriculoventricular rhythm, with  $Av-Sa=0.022$ ,  $As-Vs=0$ , cycle=0.68. The records were made only after the interruptions were completed.

EXPERIMENT 15.—Normally  $Sa-Av=0.022$ ,  $Sa-Ra=0.013$ ,  $As-Vs=0.08$ , cycle=0.29. A cut was made along the auricular border of the sulcus;  $Sa-Ra$  increased to 0.02, with no further change. A cut made along the intercaval border of the sulcus produced auriculoventricular rhythm with  $Av-Sa=0.026$ ,  $Vs-As=0.03$ , cycle=0.40. The records were made only after the interruptions were completed.

EXPERIMENT 16.—Normally  $Sa-Av=0.03$ ,  $Sa-Ra=0.014$ ,  $As-Vs=0.10$ , cycle=0.42. A cut was made along the auricular border of the sulcus;  $Sa-Ra$  increased to 0.028, with no further change. A cut was made along the intercaval border of the sulcus; the pacemaker removed to the lower end of the sino-auricular node;  $As-Vs=0.09$ , cycle=0.47. A ligature was tied across below the sulcus. Auriculoventricular was established, with  $Av-Sa=0.019$ ,  $As-Vs=0.07$ , cycle=0.48. There was no evidence of partial block before or during the establishment of this rhythm.

EXPERIMENT 17.—Normally  $Sa-Av=0.024$ ,  $Sa-Ra=0.016$ , cycle=0.30. A cut was made along the intercaval border of the sulcus, with no change. A ligature was tied across the sulcus above the node, with no change. Ligatures were tied across the sulcus below the node. The first of these caused no change; the second produced auriculoventricular rhythm, with  $Av-Sa=0.031$ ,  $Av-Ra=0.038$ ,  $As-Vs=0$ , cycle=0.36. No evidence of block in passage into this rhythm.

EXPERIMENT 18.—Normally  $Sa-Av=0.035$ ,  $Sa-Ra=0.03$ ,  $As-Vs=0.14$ , cycle=0.29. A cut was made along the upper half of the intercaval border of the sulcus. There was a transitory period of coronary sinus rhythm with  $Sa-Av=0$ ,  $As-Vs=0.09$ , cycle=0.33. Later there was a return to normal rhythm. A cut was made along the lower half of the intercaval border of the sulcus, with no change. A ligature tied across the sulcus below the node produced auriculoventricular rhythm with  $Av-Sa=0.031$ ,  $Av-Ra=0.047$ ,  $As-Vs=0.04$ , cycle=0.37. There was no evidence of block in the development of this rhythm.

EXPERIMENT 19.—In this experiment galvanometer records were made only at times to determine with certainty the position of the pacemaker. Frequent records were made of the systole of the right auricle and ventricle on a Hürthle kymographion. Ligatures were laid across the sulcus above and below the node. The upper one was tied without result; the lower one produced auriculoventricular rhythm. The  $As-Vs$  interval shortened from 0.11 to 0.03; the cycle lengthened from 0.38 to 0.47. This ligature was now removed and normal rhythm returned, with  $As-Vs=0.12$ , cycle=0.38.

With the ligature above the node remaining tied, the lower ligature was removed and a clamp was inserted along the intercaval borders of the sulcus. Putting it in position produced no effect, nor did screwing it down tight. The clamp was now loosened and the ligature laid and tied across the sulcus below the node. This produced auriculoventricular rhythm with recovery of sino-auricular rhythm after removal. Later auriculoventricular rhythm was again established. Sino-auricular rhythm returned on application of warmth to the head of the sino-auricular node. This rhythm remained after tying the ligature again below the node, but auriculoventricular rhythm resulted when the clamp along the intercaval border was tightened. Loosening the clamp, removing upper and lower ligatures and warming the node resulted in a return of sino-auricular rhythm. Auriculoventricular rhythm was again produced by replacing the ligatures and gradually tightening the clamp. Finally, application of heat to the node caused return of sino-auricular rhythm, with ligatures and clamp in place. Transitory auriculoventricular rhythm with recovery was now produced several times and at will by traction on one or the other of the ligatures. Records were taken throughout the above procedures. Usually the transition from sino-auricular to auriculoventricular rhythm occurs within one beat, in other cases the changes were gradual. None of the records give any indication whatever of partial block at any time.

EXPERIMENT 20.—This experiment is similar as to the procedures carried out and the results obtained as the last, except that the condition of complete sino-auricular block during the periods of auriculoventricular rhythm was



proved. A cut was first made along the auricular border of the sulcus. This was without effect. Ligatures were tied across above and below the node without change. A clamp was now placed along the intercaval border, connecting the ligatures above and below and gradually tightened. Auriculoventricular rhythm resulted. The *As-Vs* interval shortened from 0.12 of a second to 0. The cycle lengthened from 0.40 to 0.53. On loosening the clamp sino-auricular rhythm returned. The transition from sino-auricular to auriculoventricular rhythm was abrupt, within one beat. Following the normal cycles of 0.40, there occurred a pause of 0.67 of a second and auriculoventricular rhythm set in at once with *As-Vs* of 0, cycle of 0.53. The production of and recovery from auriculoventricular rhythm was brought about several times by tightening and loosening the clamp. Later, after loosening the clamp, auriculoventricular rhythm continued.

Stimulation of the right vagus nerve in this condition produced a transitory return of sino-auricular rhythm lasting for seven cycles, then return of auriculoventricular rhythm. The inotropic effect of the vagus on the auricle made it evident visually that the region of the sino-auricular node was beating at a much faster rate than the auricles or ventricles, and that we were dealing either with a definite partial or complete sino-auricular heart block. Electrodes were therefore placed in the usual position and the above procedures duplicated. These records show (Figs. 4 and 5), reading from top to bottom, (1) time in one-fifth second intervals; (2) signal pen; (3) galvanometer thread connected to electrodes on sino-auricular node and right auricle; upward movement of the curve indicating negativity in the node; (4) systoles of the right auricle, and (5) right ventricle, the systole being indicated by down strokes in each case. Figure 4 shows the transition from sino-auricular rhythm to auriculoventricular rhythm with complete sinoventricular block, as a result of tightening the clamp along the intercaval border. The transition occurs within one cycle. It is evident that the sino-auricular node is giving evidence of its activity here independently of the activity of any other part of the heart. The normal cycle length averages 0.42 of a second and the sino-auricular node continues to discharge at this rate during the auriculoventricular rhythm. The rate of discharge of the auriculoventricular node, which is now acting as pacemaker for the heart, is given by a cycle length averaging 0.55 of a second.

Figure 5 shows the effect of vagus stimulation. The clamp was tightened just enough to produce auriculoventricular rhythm before this procedure. The sino-auricular rate of discharge before the vagus stimulation was indicated by a cycle length of 0.44 of a second. The auriculoventricular node discharged at intervals of 0.54 of a second. During the vagus stimulation there was a greater depressing effect evident on the discharge of the auriculoventricular than on the sino-auricular node, due, no doubt, to the fact that the ligatures and clamp had interfered with a part of the connections of the latter with the nerve. There is no definite relation between the sinoventricular and auriculoventricular impulses, during or before the stimulation, and the condition is evidently one of complete block.<sup>13</sup> The above procedures were repeated several times: normal sino-auricular rhythm, production of auriculoventricular rhythm by gradually clamping, the influence of vagus stimulation, and finally the return of sino-auricular rhythm as a result of loosening the clamp. At no time was there any evidence of partial block.

13. It may be noted that in this and in subsequent periods of vagus stimulation, while auriculoventricular rhythm and complete sinoventricular and sino-auricular block persisted, the auriculoventricular interval lengthened from 0 to an average of about 0.08 of a second. Lengthening of the *As-Vs* interval on vagus stimulation during auriculoventricular rhythm does not necessarily mean, therefore, the return of sino-auricular rhythm, as has been generally assumed. A further example of this is given in Figure 6.



EXPERIMENT 21.—This experiment was similar to the last. A cut was made along the auricular border of sulcus, ligatures were applied above and below the sino-auricular node, and a clamp along the intercaval border of the sulcus. Coronary sinus rhythm was produced by tightening the clamp. The galvanometer records showed no indication of activity of the sino-auricular node independent of that of the coronary sinus region. The apparently dropped beats observed in the records of the mechanical systoles were shown by the galvanometer records to be pauses following extrasystoles.

EXPERIMENT 22.—Cuts were made along the auricular border of the sulcus and across the sulcus above and below the sino-auricular node without producing loss of function of the node as pacemaker. A clamp was now applied along the intercaval borders of the sulcus and was gradually tightened. The records made during this procedure showed no change until the sudden occurrence of auriculoventricular rhythm. *As-Vs* shortened from 0.08 to 0; the cycle lengthened from 0.40 to 0.50. There was no indication of partial block. The galvanometer records show no indication of activity of the sino-auricular node independent of the auriculoventricular node during the auriculoventricular rhythm.

EXPERIMENT 23.—Cuts along the auricular border of the sulcus, above and below the node, caused no change in the pacemaker. Clamping along the intercaval border of the sulcus produced auriculoventricular rhythm. Normal rhythm returned on loosening the clamp and warming the sino-auricular node. Auriculoventricular rhythm was produced by again tightening the clamp. The sino-auricular rhythm was restored by loosening the clamp and warming the node or by mild vagal stimulation. These experiences were repeated a number of times with records made during all procedures. There was no indication at any time of partial sino-auricular block.

EXPERIMENT 24.—This was practically identical in procedures and results to the last experiment.

EXPERIMENT 24.—This was practically identical in procedures and results conclusively that complete sinoventricular and sino-auricular block may be associated with auriculoventricular rhythm. Auriculoventricular rhythm was produced by a cut along the auricular border of the sulcus and ligatures above and below the node. Tying permanently one of these ligatures and traction on the other was sufficient to cause auriculoventricular rhythm to develop, sino-auricular rhythm returning on cessation of the traction. Galvanometer records showed an independent and more rapid discharge of the sino-auricular node during the periods of auriculoventricular rhythm. Numerous records made during the transition from one type of rhythm to the other showed no evidence at any time of partial block. During the auriculoventricular rhythm, vagus stimulation caused a greater slowing of the discharge from the auriculoventricular than the sino-auricular node.

EXPERIMENT 26.—The coronary sinus rhythm was produced by a cut along the auricular border, ligatures above and below the node and clamping along the intercaval border of the sulcus.

EXPERIMENT 27.—This was similar to last experiment. The coronary sinus rhythm was produced by a cut along the auricular border and ligatures above and below the node.

EXPERIMENT 28.—This was similar to last experiment. The auriculoventricular rhythm was produced by ligatures above and below the node and clamping along the intercaval border.

EXPERIMENT 29.—Tying a ligature above the node caused a lengthening of the *Sa-Av* conduction time from 0.019 to 0.023. After four cycles there were six cycles of auriculoventricular rhythm in which  $Av-Sa = 0.007$  of a second.

and during which  $As-Vs$  shortened progressively from 0.11 to 0.02 of a second. These were followed by a sudden return of sino-auricular rhythm with  $Sa-Av=0.04$  of a second, and a gradual lengthening of  $As-Vs$ . The change in the seat of the pacemaker occurred within one beat, while the  $As-Vs$  shortened gradually and also returned only gradually to its normal length on the restoration of sino-auricular rhythm. Tying the ligature across the sulcus below the node produced permanent auriculoventricular rhythm with  $As-Vs=0$ . The cycle lengthened from 0.32 to 0.39 of a second.  $Av-Sa=0.036$  of a second. Vagus stimulation was without influence until the two ligatures were removed, when a transitory period of return of sino-auricular rhythm occurred, with  $Sa-Av=0.05$ ,  $As-Vs=0.08$  of a second. There was no evidence of partial block during the transitions from one type of rhythm to the other.

EXPERIMENT 30.—Normally  $Sa-Av=0.031$ ,  $As-Vs=0.19$ , cycle=0.35 of a second. A ligature was laid across the sulcus above the sino-auricular node. The first cycle after tying this ligature is an auricular extrasystole. The third cycle shows  $Sa-Av=0.035$ , while  $As-Vs$  has shortened to 0.027 of a second. The fourth cycle shows the same  $Sa-Av$ , while  $As-Vs$  has become zero. The fifth cycle shows  $Av-Sa=0.032$ ,  $As-Vs=0$ . The sixth to the thirteenth cycles show  $Av-Sa=0.064$ ,  $Vs-As=0.023$ . The fourteenth cycle is an ectopic ventricular beat. The fifteenth cycle shows  $Sa$  and  $Av$  simultaneous,  $As$  and  $Vs$  simultaneous. The sixteenth cycle after the ligature was tied shows  $Sa-Av=0.04$ ,  $As-Vs=0$ . In the succeeding cycles,  $Sa-Av$  remains about the same,  $As-Vs$  gradually lengthens through succeeding cycles to 0.014, 0.051, 0.086, 0.125, 0.150, 0.160, 0.166 of a second. The twenty-fifth cycle after the ligature was tied shows  $Sa-Av=0.05$ ,  $As-Vs=0.17$  of a second.

Ligaturing across the sulcus above the head of the node thus produced transitory auriculoventricular rhythm lasting for fourteen cycles, with return to sino-auricular rhythm with increased  $Sa-Av$  conduction time. In the transition into auriculoventricular rhythm the change from  $Sa-Av$  to  $Av-Sa$  was abrupt, the change in  $As-Vs$  more gradual. This is even more marked during the return to normal sino-auricular rhythm. Here the change from  $Av-Sa$  to  $Sa-Av$  occurred within two cycles, while  $As-Vs$  showed a gradual and progressive increase extending over seven cycles.

Ligaturing across the sulcus below the node caused no change except a moderate shortening of  $As-Vs$ . After this ligature was tied,  $Sa-Av=0.05$ ,  $As-Vs=0.13$ , cycle=0.38. Clamping along the intercaval border of the sulcus caused no further change.

EXPERIMENT 31.—Normally  $Sa-Av=0.014$ ,  $As-Vs=0.09$ , cycle=0.41. Ligation above the head of the sino-auricular node produced transitory auriculoventricular rhythm with changes similar to those described in the last experiment.

EXPERIMENT 32.—The changes in  $Sa-Av$  during the establishment of transitory auriculoventricular rhythm as a result of ligation above the sino-auricular node differ from those in Experiment 30, in that they occur gradually and not abruptly. These changes in successive cycles subsequent to the ligation are as follows:  $Sa-Av=0.010$ , 0.007, 0.006, 0.005;  $Av-Sa=0.004$ , 0.007, 0.014, 0.010, 0.010, 0.010, 0.006, 0.000;  $As-Vs=0.090$ , 0.073, 0.072, 0.073, 0.060, 0.058, 0.057, 0.063, 0.063, 0.063, 0.068, 0.078;  $Sa-Av=0.015$ , 0.017, 0.020;  $As-Vs=0.088$ , 0.091, 0.010.

Ligation across the sulcus below the node produced at first lengthening of  $Sa-Av$  to 0.035, and within one minute after the ligation there was auriculoventricular rhythm with  $Av-Sa=0.027$ ,  $As-Vs=0$ , cycle=0.44. Vagus stimulation failed to produce a return of sino-auricular rhythm, but caused the occurrence of numerous idioventricular beats.

EXPERIMENT 33.—Normally  $Sa-Av=0.036$ ,  $As-Vs=0.18$ , cycle=0.36. During ligation above the head of the node there occurred successively two normal



beats, one ventricular extrasystole, one cycle, in which  $Sa$  and  $Av$  were simultaneous and  $As-Vs=0.045$ , one ventricular extrasystole, one normal beat, one ventricular extrasystole, one auriculoventricular beat with  $Av-Sa=0.03$ ,  $As-Vs=0$ , one ventricular extrasystole, two normal beats, one ventricular extrasystole with retrograde conduction ( $Vs-As=0.08$ ), one ventricular extrasystole. Then normal cycles continued with  $Sa-Av=0.045$ ,  $As-Vs=0.16$ , cycle  $=0.37$ .

Ligation of sulcus below the sino-auricular node now caused successively one cycle with  $Av-Sa=0.07$ ,  $Vs-As=0.065$ , one cycle with  $Sa-Av=0.02$ ,  $As-Vs=0$ , one cycle with  $Sa-Av=0.03$ ,  $As-Vs=0.135$ , then continuance of normal cycles with  $Sa-Av=0.04$ ,  $As-Vs=0.14$ , cycle  $=0.43$ .

Clamping the tissue along the intercaval borders of the node produced auriculoventricular rhythm with  $Av-Sa=0.019$ ,  $As-Vs=0.10$ , cycle  $=0.49$ . The onset of this rhythm was abrupt. No further influence was exerted by a cut along the auricular border of the sulcus.

EXPERIMENT 34.—Normally  $Sa-Av=0.022$ ,  $As-Vs=0.15$ , cycle  $=0.54$ . Ligation across the sulcus below the node produced temporary reversal of the galvanometer on the sino-auricular node, then  $Sa-Av$  increased to 0.05,  $As-Vs$  increased to 0.17, and the cycle to 0.50. Later  $Sa-Av$  shortened to 0.03. Ligation across the sulcus above the head of the node produced several ectopic beats; then  $Sa-Av$  increased to 0.045,  $As-Vs=0.18$ , cycle  $=0.47$ . Clamping along the intercaval border of the sulcus increased  $Sa-Av$  to 0.05, and one minute later abrupt onset of auriculoventricular rhythm occurred, with  $Av-Sa=0.014$ ,  $As-Vs=0.13$ , cycle  $=0.62$ . The clamp was now removed and placed along the auricular border of the sulcus. Following the loosening of the clamp, sino-auricular rhythm returned, with  $Sa-Av=0.03$ ,  $As-Vs=0.17$ , cycle  $=0.47$  (Fig. 1). Clamping the auricular border of the sulcus produced sino-auricular and sinoventricular heart block. The results are shown in Figures 2 and 3. In the record (Fig. 2) the upper galvanometer curve records the activity of the sino-auricular node, the lower the auriculoventricular node. The upper tambour curve records, by its downstroke, mechanical systole of the right auricle; the lower, mechanical systole of the right ventricle. It will be seen that each period of activity in the sino-auricular node is followed by activity in the auriculoventricular node, but progressively later. The  $Sa-Av$  conduction period thus gradually lengthens until complete failure of conduction occurs. Three records made in the course of three minutes during this condition show that usually it was every sixth sino-auricular impulse which was blocked. Occasionally every fifth or every sixth sino-auricular impulse failed to be conducted. The condition may therefore be termed 6 to 5 and 5 to 4 partial sino-auricular and sinoventricular block. There was a small open path of tissue about 4 mm. wide between the lower end of the clamp and the ligature across the lower part of the sulcus. Clamping this region did not increase the degree of block, but produced prompt auriculoventricular rhythm.

EXPERIMENT 35.—Normally  $Sa-Av=0.013$ ,  $As-Vs=0.10$ , cycle  $=0.36$ . Tying a ligature across the sulcus below the node caused at first three ectopic beats, then normal cycles, but with increased  $Sa-Av$  conduction (0.024). This returned after removal of this ligature, to 0.015 of a second. Ligation across the sulcus above the node lengthened  $Sa-Av$  to 0.04, but caused no further change. Tying a ligature below the node again produced in succession one normal cycle with  $Sa-Av=0.036$ ,  $As-Vs=0.12$ ; one auriculoventricular cycle with  $Av-Sa=0.32$ ,  $As-Vs=0.048$ ; one cycle with  $Sa$  and  $Av$  simultaneous,  $As-Vs=0.08$ ; and then normal cycles with  $Sa-Av=0.036$ ,  $As-Vs=0.105$ , cycle  $=0.38$ . One-half minute later the condition was the same, one minute later  $Sa$  and  $Av$  were found to be simultaneous;  $As-Vs=0.035$ , cycle  $=0.45$ . The next record,



one-half minute later, showed  $V_s-A_s=0.02$ , cycle=0.53, and one minute later a definite auriculoventricular rhythm was evident with  $Av-Sa=0.02$ ,  $As-V_s=0.03$ , cycle=0.50. The change to definite auriculoventricular rhythm was thus gradual. At no time was there evidence of partial block. The auriculoventricular rhythm was permanent. Vagus stimulation temporarily lengthened  $As-V_s$  to 0.085, but caused no change in the seat of impulse formation.<sup>18</sup>

## THE OCCURRENCE OF NUCLEAR CHANGES IN THE RED BLOOD CELLS FOLLOWING SPLENECTOMY\*

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Much attention has recently been given to the rôle and function of the spleen. Its relation to the destruction of red cells and to the catabolism of hemoglobin has been particularly emphasized, whereas much less has been noted concerning the function which it may play in controlling or affecting the histogenesis of these cells. The view that the histogenesis of red corpuscles is in some way dependent on splenic function is based primarily on the observation that following splenectomy the red blood cells show nuclear particles (so-called Howell-Jolly bodies), which were not present before the operation.

Howell<sup>1</sup> in 1890 noted that following hemorrhage the erythrocytes of cats often showed small bodies, in many respects resembling nuclear material. These were described as "single, good-sized pieces of nuclear material, too large to be called granules, but having the shape and appearance of large nucleoli, which always lay imbedded in the periphery of the spherical corpuscle."

These bodies were also studied by Schmauch<sup>2</sup> and by Morris.<sup>3</sup> Although they were found to resemble nuclear bodies in that they were oval or round and took the nuclear stains, they differed from nuclear material in that they did not stain after fixation with ethyl alcohol, with methyl alcohol, with alcohol and ether, with alcohol and formaldehyde or with heat for thirty seconds at the spheroidal point for water. Fixation with heat and shorter fixation with alcohol and formaldehyde preserved these bodies fairly well.

Similar bodies were found by Schmauch<sup>2</sup> in 1899 in cats' blood after pyrocin poisoning and after the administration of the extract of *Bothriocephalus latus*; also by Schmidt<sup>4</sup> in 1902 in new-born rats, adult

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1. Howell: The Life History of the Formed Elements, Especially the Red Corpuscles, Jour. Morphology, 1890, **4**, 57.

2. Schmauch: Ueber endoglobulare Körperchen in den Erythrocyten der Katze, Virchows Arch. f. path. Anat., 1899, **156**, 201.

3. Morris: Note on the Occurrence of Howell's Nuclear Particles in Experimental Anemia of the Rabbit and in Human Blood, Bull. Johns Hopkins Hosp., 1907, **18**, 198.

4. Schmidt: Experimentelle Beiträge zur Pathologie des Blutes, Jene, 1902, cited by Pol.

mice and in rabbits after phenylhydrazin poisoning; by Pol<sup>5</sup> in rabbits after phenylhydrazin poisoning; by Jolly<sup>6</sup> in 1905 in normal pregnant rats, in new-born mice and white rats, as well as the embryos of white rats and mice; by Morris<sup>7</sup> in 1907 after pyrodin poisoning in rabbits. Morris<sup>7</sup> also observed the bodies in nine cases of pernicious anemia, two cases of anemia of infants, two cases of secondary anemia and three cases of chronic myeloid leukemia. Naegeli<sup>8</sup> in 1908 also saw these particles in the blood of pernicious anemia, in the embryos of animals and in different forms of anemia. Cabot<sup>9</sup> pictures multiple nuclear particles in the bone marrow cells which were not found in the peripheral blood. Richards and Johnson<sup>10</sup> and Huber<sup>11</sup> observed these same bodies in congenital hemolytic jaundice.

In all of these cases nuclear particles were found only occasionally, except in the cats' blood. Although some of the bodies found in the cats' blood did not conform entirely with the reactions of nuclear material, it seems to be generally accepted that they are true nuclear particles or directly derived from nuclei.

In 1909 Morris<sup>7</sup> called attention to these nuclear particles in the blood of a patient whose spleen had been removed, but at that time he did not attribute their occurrence to the removal of the spleen. In 1908 Schur<sup>12</sup> found many nuclear particles in the erythrocytes of a patient who showed a markedly atrophic spleen at necropsy. O. Roth<sup>13</sup> in 1912 observed nuclear particles in the red blood cells of a patient whose spleen had been removed twelve years previously, and he estimated that there were as many as 20,000 per cubic millimeter. Mosse<sup>14</sup> in 1913 found the "Jolly-körperchen" regularly after splenectomy.

5. Pol: Studien zur pathologischen Morphologie der Erythrocyten; Thesis, Heidelberg, 1905, cited by Morris.

6. Jolly: Sur la formation des globules rouges des mammifères, *Compt. rend. Soc. de biol.*, 1905, **68**, 528; Sur l'évolution des globules rouges dans le sang, *ibid.*, 593.

7. Morris: Nuclear Particles in Erythrocytes, *THE ARCHIVES INT. MED.*, 1909, **3**, 93.

8. Naegeli: Ueber Basophile Granulations der Erythrocyten bei Embryonen, *Folia Haemat.*, 1908.

9. Cabot: Clinical Examination of the Blood, 1908.

10. Richards and Johnson: Study of a Case of Congenital Hemolytic Jaundice, *Jour. Am. Med. Assn.*, 1913, **61**, 1586.

11. Huber: Ueber die Blutveränderungen bei Icterus Haemolyticus, *Berl. klin. Wchnschr.*, 1913, **50**, 681.

12. Schur: Ueber eigenartige basophile Einschlüsse in den roten Blutkörperchen bei einem Fall von Abgelaufenem Morbus Basedowii mit nachfolgender schwerer macrocytischer anämie, *Wien. med. Wchnschr.*, **58**, 511.

13. O. Roth: Ueber merkwürdige Erythrocyten Einschlüsse bei einem Fall von Milzextirpation, *Ztschr. f. klin. Med.*, 1912, **76**, 23.

14. Mosse, M.: Zur Lehre von den Krankheiten mit gesteigerter Hämolyse: (a) Pigment Cirrhose; (b) Milzextirpation bei Pernicios-Hämolytischer Anämie, *Berl. klin. Wchnschr.*, 1913, **1**, 2088.



Von DeCastello<sup>15</sup> observed similar bodies in about 5 per cent. of the red blood cells one-half year after splenectomy. He, with Riedel, found them also in four splenectomized dogs. Hirschfeld and Weinert<sup>16</sup> in 1914 found nuclear particles in guinea-pigs after splenectomy and further observed that after bleeding these occurred in much larger numbers when the spleen had been removed. They quote Huber as having found them in rats and guinea-pigs after splenectomy. In addition they studied the blood of fourteen patients from whom the spleens had been previously removed. Six of these patients had had ruptured spleens, while the remainder had suffered from splenic anemia, miliary tuberculosis of the spleen, pseudoleukemia infantum, Banti's disease or icterus with splenic tumor. Although the examinations were made from one to seven years after the operation, the blood which might otherwise be normal showed Jolly-körperchen in each case. Hirschfeld with Klemper<sup>17</sup> noted their presence four hours after splenectomy. Morris<sup>18</sup> in 1915 reported three cases in which nuclear particles were found after extirpation of the spleen. Barron<sup>19</sup> in 1915 found "Howell-Jolly" bodies in one case of splenectomy for pernicious anemia in which none were found before the operation. Lee, Vincent and Robertson<sup>20</sup> in 1915 reported five instances of splenectomy in cases of pernicious anemia in two of which the "Howell-Jolly" bodies were found the day following the operation and on the second day in the other three cases. They remained numerous from two to three months after the operation, despite the fact that the normoblasts and megaloblasts disappeared from the blood and the destruction of red blood cells had decreased or returned to normal limits. Hirschfeld<sup>21</sup> in 1915 found constantly the same nuclear changes in the red blood cells following splenectomy in various blood diseases. And I have recently found them in three cases of pernicious anemia without operation and in one case of lymphatic leukemia, and in one case of severe secondary anemia, but only after prolonged search.

In most cases no mention is made of their occurrence before the

15. Von Decastello: Ueber den Einfluss der Milzextirpation auf die Perniciöse Anämie, Deutsch. med. Wchnschr., 1914, **40**, 692.

16. Hirschfeld and Weinert: Klinische und experimentale Untersuchungen über den Einfluss der Milz auf die erythroplastische Thätigkeit des Knochenmarks, Berl. klin. Wchnschr., 1914, **51**, 1026.

17. Hirschfeld and Klemper: Milzextirpation zur Behandlung der perniciosen Anämie, Therap. d. Gegenw. 1913, **54**, 385.

18. Morris: The Occurrence of Nuclear Particles in Erythrocytes Following Splenectomy, THE ARCHIVES INT. MED., 1915, **15**, 514.

19. Barron: Pathology of Pernicious Anemia, Journal-Lancet, 1915, **35**, 452.

20. Lee, Vincent and Robertson: Immediate Results of Splenectomy in Pernicious Anemia, Jour. Am. Med. Assn., 1915, **65**, 216.

21. Hirschfeld: Ueber die Functionem der Milz, Deutsch. med. Wchnschr., 1915, **41**, 1129.

operations, presumably because until recently attention has not especially been called to their constant appearance in such large numbers immediately following splenectomy.

The following cases of splenectomy were studied with special reference to the changes which occurred in the red blood cells:

CASE 1 (15-1508) (an atypical case of pernicious anemia with jaundice and ascites; marked improvement after splenectomy).—A Hungarian, aged 35, entered the University Hospital Oct. 26, 1915, complaining of weakness, loss of appetite, bloating and swelling of the feet. The family and personal history were unimportant. The present illness began in December, 1914, with weakness and loss of appetite. There was a marked improvement up to May, 1915, when a relapse occurred, with jaundice and swelling of the abdomen and feet. During his first stay in the hospital there were occasional sharp pains which radiated to the left back.

On examination the patient showed a markedly sallow complexion, edema of the ankles, puffiness about the eyes and signs of ascites. There was no general adenopathy. The spleen could be felt, but the size of the liver could not be definitely determined. There were marked signs of anemia; other blood changes will be discussed later.

The patient improved markedly, and left the hospital Dec. 9, 1915. He reentered on Jan. 29, 1916. In the interim his appetite had become worse, the ascites and edema more marked; there had developed a constant pain on both sides of the upper abdomen and the jaundice had gradually deepened. The anemia, however, had not grown worse. At no time were there any definite neurologic disturbances. On account of the ascites the size of the liver and spleen could not be definitely determined. The Wassermann was negative. The urine gave a positive test for urobilinogen on the first entrance and a very marked positive test on the second entrance. There was an achlorhydria, and no occult blood or parasitic ova were found in the stools.

The blood, as indicated in the table, showed a marked anemia, with a high color index and changes in the erythrocytes resembling those of pernicious anemia. There were, however, fewer macrocytes than usually occur in pernicious anemia.

The spleen was removed by Dr. Darling on Feb. 3, 1916. At the operation a large amount of bile-stained ascitic fluid was found. The liver was somewhat enlarged, very firm and slightly paler than normal. The head of the pancreas felt larger and firmer than normal. The spleen was about three times the normal size. The pathologic examination by Dr. Warthin showed, "increase of stroma, marked passive congestion, all the blood spaces dilated, splenic follicles large, but the pulp showing rather marked lymphoid exhaustion. There were no myeloid changes, but the spleen looked as in early Banti's disease."

The convalescence was uneventful except for a slight fever from the fifth to the eighth postoperative day. The jaundice and edema disappeared after about three weeks. Urobilinogenuria remained markedly positive for thirty-one days and then disappeared.

CASE 2 (16-485) (a case of pernicious anemia with edema, but without marked signs of hemolysis).—An American woman, aged 48, entered the hospital April 1, 1916, complaining of general weakness, swelling of the feet and ankles. The family history was unimportant. The patient had always enjoyed good health up to that illness, which began insidiously with weakness and indefinite aching pains in the arms and shoulders, but without paresthesia. A few weeks prior to the entrance to the hospital the feet and ankles began to swell. There had been considerable nausea and for a time a severe diarrhea.

On examination a pale waxy color was noted, no adenopathy, and a very slight edema of the ankles. The liver was not enlarged, but the spleen was

# RESULTS OF A SERIES OF BLOOD EXAMINATIONS IN CASES 1, 2 AND 3

Date	Red Blood Cells	White Blood Cells	Hemoglobin	Neutrophil polymorphonuclears	Eosinophil polymorphonuclears	Basophil polymorphonuclears	Large Lymphocytes	Small Lymphocytes	Large Mononuclears	Transferrons	Myelocytes	Megakaryoblasts	Normoblasts	Nuclear Particles per C.Mm.
Case 1														
10/30/15	2,150,000	6,700	42.5	76	0	2.5	3	11.5	2	3	0	0	0	.....
12/ 5/15	3,600,000	3,850	65	....	....	....	....	....	....	....	....	....	....	.....
2/ 3/16	3,450,000	3,970	63	64.5	0.5	1.5	6	10	8.5	9	6	0	1	None
2/ 4/16	2,950,000	10,750	65	82	0	0	0	0.5	8.5	9	0	0	8	13,800
2/ 5/16	2,610,000	12,950	66	69.5	0	0	1.5	4.5	12	12.5	0	0	9	48,000
2/ 6/16	3,100,000	12,850	64	80	1.5	0	1	0.5	5	12	0	0	7	18,000
2/ 7/16	3,150,000	14,500	66	61	1	0	6	1	10	21	0	0	18	21,700
2/ 8/16	3,050,000	21,350	66	66.5	0.5	0	3	1	8	21	0	0	6	41,400
2/ 9/16	2,850,000	24,100	62	77	0.5	0	1	0.5	6	15	0	0	8	31,700
2/10/16	2,790,000	27,500	60	82	2	0	1	1	2.1	12	0	0	7	24,600
2/11/16	2,880,000	24,200	62	85.5	0	0	0.5	1.5	1.5	11	0	0	5	18,600
2/12/15	2,680,000	20,000	58	79	1.5	1	1.5	0	5	12	0	0	5	28,400
2/14/16	2,810,000	23,700	58	78.5	4	1	2.5	0.5	6	7.5	0	0	3	19,200
2/15/16	3,100,000	82,100	56	77	1.5	0.5	3	5	3	10	2	0	2	41,500
2/16/16	3,050,000	21,600	62	75	0	0	2.5	6.5	3	13	0	0	5	32,400
2/17/16	3,170,000	17,000	58	76	2	0.6	2	1	5.6	12.4	0	0	5	14,800
2/18/16	3,010,000	16,350	62	78.6	1	0.3	2.4	2	4.6	11.1	0	0	2	14,100
2/20/16	2,950,000	17,850	63	83.4	1	0.9	3.3	2.2	2.1	7.1	0	0	4	21,500
2/22/16	2,970,000	17,100	62	78	1	0.8	2	4.2	6	8	0	0	2	39,000
2/24/16	2,790,000	16,150	61	73.5	1.5	0.5	4.5	2	7	11	0	0	0	33,700
2/27/16	2,920,000	16,000	67	79	0	0	2	8	8	8	0	0	0	44,000
3/ 2/16	3,080,000	16,000	67	67	4	0.5	2.5	6	7	13	0	0	5	36,500
3/ 5/16	3,350,000	14,950	68	59	0	0	4	0	25	14	0	0	2	28,000
3/ 8/16	3,340,000	17,700	68	79	1	1.5	5.5	1.5	8.5	8	0	0	1	23,000



3/12/16	3,300,000	15,800	68	68	1	0	6	2	11	10	0	0	0	0	30,000
3/15/16	2,750,000	17,000	59	65.5	2.5	0	8.5	7.5	7	8	0	4	0	0	30,700
3/19/16	2,900,000	15,650	65	41	3	1.5	10	15.5	21.5	7.5	0	2	0	0	38,300
3/27/16	3,190,000	15,000	62	45	1	0	7	2.5	37	7.5	0	2	0	0	40,000
3/30/16	3,530,000	17,000	69	64.5	2.5	0	6.5	4	18	5	0	1	0	0	30,600
Case 2															
4/14/16	2,700,000	6,200	....	65	0.5	0	14	12	6.5	2	3	0	3	0	None
4/25/16	2,500,000	5,400	53	46	2.5	0	14.5	12.5	20.5	4	0	0	0	0	None
4/27/16	2,680,000	5,300	52	42.5	4	0	16	21	9	2.5	0	0	0	0	None
4/28/16	2,900,000	21,750	49	30	0	0	3	2	3	2	0	1	6	6	2,300
4/29/16	2,800,000	27,600	49	91	0	0	3	1	2.5	2.5	0	0	6	6	4,010
4/30/16	2,700,000	28,000	50	88.5	1	0	4	5.5	2	1	0	0	5	5	2,800
5/1/16	2,550,000	18,900	49	89	1	0	0.5	9	0.5	0	0	0	9	9	3,200
5/2/16	2,610,000	16,000	50	88.5	0	0	4.5	3.5	1.5	2	0	0	11	11	3,360
5/3/16	2,700,000	26,150	49	91.5	0	0	4	4	0	0.5	0	0	10	10	4,000
5/5/16	2,500,000	15,000	43	84.5	0.5	0.5	3	6.5	4	1	1	4	35	35	12,200
5/6/16	2,540,000	11,450	46	66.5	2.5	1	9	11	5	5	0	1	15	15	11,700
5/8/16	2,350,000	12,950	42	51.5	2	2.5	13	12	10	10	1	1	12	12	23,200
5/10/16	2,610,000	10,500	47	61	4	0.5	8	17	4	5.5	4	1	16	16	12,300
5/12/16	2,740,000	11,300	52	67	1	1	7.5	8	9	6.5	0	0	9	9	21,300
5/14/16	2,600,000	10,000	50	56	1.5	0.5	7.5	17.5	5.5	11.5	4	0	9	9	9,250
5/16/16	2,690,000	9,850	55	59	2	0.5	8	6.5	12	12	0	0	1	1	23,500
5/18/16	2,930,000	10,000	56	56	3.5	1.5	9	4	15	11	0	0	5	5	10,900
5/21/16	2,690,000	11,500	57	61	5	1	4	4	13.5	11.5	0	0	6	6	24,800
5/23/16	2,800,000	10,050	57	59	0.5	0.5	10	5	8	17	0	0	3	3	12,500
5/26/16	2,700,000	9,900	56	38	6	1	14	6	20	15	0	0	3	3	34,000
5/31/16	3,090,000	7,100	62	33	5	2	7	21	8	24	0	0	0	0	17,700
6/3/16	3,100,000	7,900	64	43	6	2	9	15	12	13	0	0	2	2	21,000
7/13/16	3,240,000	8,000	50	50	2	0	12	14	10	12	1	0	2	2	12,000
Case 3															
5/1/16	4,500,000	6,000	75	25.5	8	1	9.5	48	3.5	4.5	0	0	0	0	4,500

definitely felt. Neurologic examination was negative. There was no urobilinogenuria. There was an achlorhydria. The blood urea was 0.025 gm. per 100 c.c. The urine and Wassermann reaction were negative, and the stools were negative for occult blood.

As will be seen from the table, the blood showed a marked anemia, with a high color index. The smears showed a decrease in platelets, some anisocytosis, a more definite macrocytosis, very few poikilocytes and an occasional normoblast and megaloblast.

Splenectomy was done by Dr. Darling, April 27, 1916. "There was no ascites, the liver was not enlarged and not nodular. The spleen was about one and one-half times the normal size. There was very little hemorrhage at the time of removal."

The pathologic examination by Dr. Warthin was as follows: "Chronic passive congestion, diffuse fibrosis, sclerosis of arterioles, follicles hyperplastic, small peritoneal cyst in capsule; no evidence of excessive hemolysis or of increased blood formation."

The immediate postoperative recovery was interfered with because of a mild pneumonia or pulmonary infarct, which developed on the fourth day, followed by a rather delayed resolution.

CASE 3.—A man, aged 37, who now is a practicing physician in good health, had his spleen removed for Banti's disease eight years ago. For the last six years his red blood cell count has been constantly about 4,500,000, and the white count between 5,000 and 6,000, with a hemoglobin of about 75 per cent.

#### CHANGES IN THE WHITE CELLS

Musser<sup>22</sup> has reviewed the literature of cases of splenectomy in which there was no primary blood disease, and he found that there was usually an immediate polymorphonuclear leukocytosis, oftentimes a lymphocytosis and in many cases a transient anemia of the secondary type. His experiments, conducted in the laboratory of R. M. Pearce, have established that after splenectomy in dogs there is (1) on a mixed diet a secondary anemia for two and one half months; (2) an immediate leukocytosis; (3) an absence of eosinophils at first, followed later by an increase; (4) a decrease and later an increase in the large mononuclear and transitional forms. The effect of splenectomy on the blood counts of our patients is shown in the table.

Case 1, following splenectomy, showed an immediate persistent leukocytosis, which was characterized by a marked increase in the polymorphonuclear neutrophils and an absolute increase in the large mononuclears and transitionals. The lymphocytes, which were absolutely decreased, and the eosinophilic and polymorphonuclear basophils, which were absent following splenectomy, later progressively increased.

In the eighth week following splenectomy there was a transient but marked increase in large mononuclears, with a decrease in the polymorphonuclear forms. These mononuclear cells were about the size of the largest polymorphonuclear, and were characterized by

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22. Musser: An Experimental Study of Changes in the Blood Following Splenectomy, *THE ARCHIVES INT. MED.*, 1912, **9**, 592.

oval, discrete nuclei, a pale, flimsy cytoplasm and numerous large azure granules. After about eight days these mononuclear cells disappeared and at the same time large numbers of degenerating cells were present. This crisis of large mononuclear cells cannot be attributed to the removal of the spleen, because in Case 2 there was a similar transient increase in the large mononuclears two days before the spleen was removed.

In Case 2 there was a more marked initial leukocytosis, which gradually decreased. As will be seen from the table, there was also an actual though variable increase in the large mononuclear and transitional forms. This was not so marked as in Case 1. There was also a marked decrease in the eosinophilic and basophilic polymorphonuclear, as well as in the large and small lymphocytes, which was followed by an increase after the first week. Possibly the mild pneumonia affected the blood picture in this case, although the formula of change after operation is similar to that noted in Case 1. The white cell count in Case 2 decreased gradually despite the intercurrent injection.

The blood platelets, which were almost absent in both patients previous to splenectomy, showed a progressive increase. This was especially marked in Case 2, in which the platelets were characterized by the presence of unusually large forms.

The blood in Case 3 was interesting from the fact that in the smear examined, eight years after splenectomy, there was a marked absolute increase in the lymphocytes.

#### CHANGES IN THE RED CELLS

Both of our cases showed a fall in the number of red blood cells and in the percentage of hemoglobin. This persisted for about two weeks after the operation.

The red cells in Case 1 became more normal in size and shape as recovery took place, while in Case 2 the macrocytosis became more marked, especially about two weeks after the operation. The changes in the red blood cells in Case 1 on the whole became less like pernicious anemia as improvement took place, although the color index remained high, while in Case 2, with unquestioned pernicious anemia, these changes were even more marked during the period of our observation. On a return examination eleven weeks after operation there existed the same changes in the size and shape of the red blood cells, although there apparently was a decrease in hemoglobin and a marked lowering of color index.

Polychromasia was more noticeable during the early weeks following splenectomy, but was not in any definite way associated with the



occurrence of nuclear particles. Together with the increase in the number of nucleated red cells, this suggests that some less mature cells were in the peripheral blood at this time.

In both patients there was an immediate increase in the number of nucleated red blood cells. For the most part they were of the normoblast type. A few typical megaloblasts were found in Case 2. In both cases the nucleated forms of red cells gradually became less numerous. During the first two weeks there was a more marked polymorphism of the nuclei of the red cell, as well as division activity, characterized chiefly by budding and fission. During this time numerous transitions between typical nuclei and the nuclear particles could be found (Figs. 5, 10, 15, 16 and 17). The larger forms of the nuclear particles were also more in evidence. It was often indeed difficult to tell just whether a cell had a true nucleus or had a nuclear particle. After about two weeks these transitional forms became rare and the smaller types of nuclear particles were usually found.

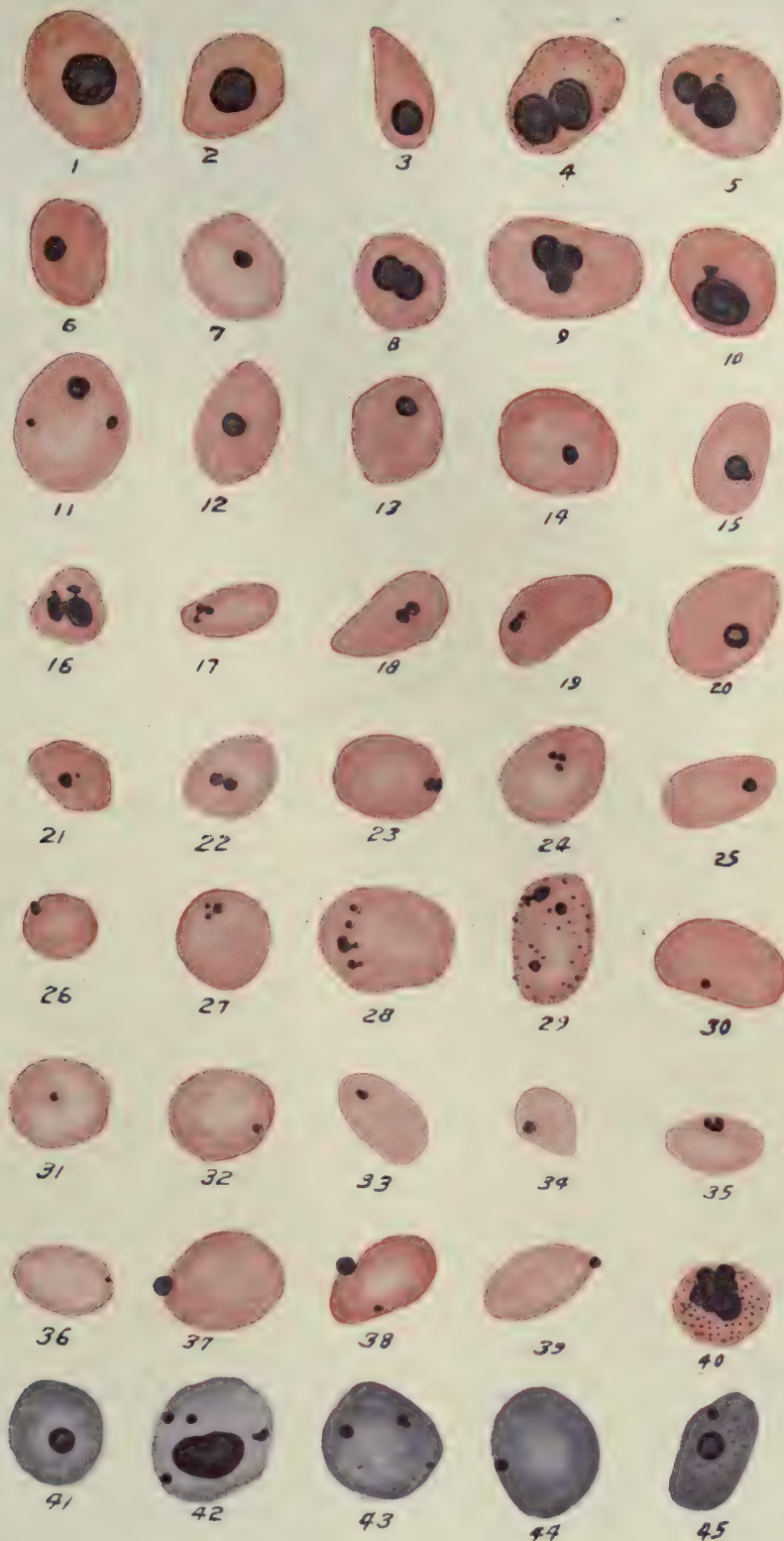
#### NUCLEAR PARTICLES FOLLOWING SPLENECTOMY

The blood in Cases 1 and 2 showed no nuclear particles in the specimens examined prior to the operation. They appeared promptly after the operation, as will be seen from the table. The number of particles was estimated in proportion to the number of white cells present.

In Case 1 twenty hours after operation there were 13,800 red blood cells per cubic millimeter, which showed nuclear particles. The number increased rapidly, the largest number being present on the second day. The number remained high without any apparent relation to the changes in the number or kind of either red or white cells.

In Case 2 nuclear particles were found on the first examination, sixteen hours after the operation. They gradually increased and were numerous on the seventh day after the end of the febrile period. Numerous other observers have found nuclear particles in large numbers immediately after splenectomy, as well as an increase in the number of megaloblasts and normoblasts. We were unable, however, to demonstrate any numerical relationship between the number of nucleated red cells and the number of nuclear particles. In fact no direct relationship could be established between the count of nuclear particles and any other changes which took place in the blood.

The nuclear particles usually look like very small discrete pycnotic nuclei, as will be seen from the accompanying illustration; a series of cells can be easily arranged which show all the transition forms from a typical normoblastic nucleus to typical nuclear particles. From our preparations it would appear that these nuclear particles arise by one of two methods: (1) A small bud may be constricted off from the



Drawn with Zeiss camera lucida,  $\frac{1}{12}$  oil immersion, No. 4 ocular. Figures 1 to 40 were stained with Wright's stain, showing transitions from the normoblast to the nuclear particles. Figures 41 to 45 were stained with methyl-green-pyronin after heat fixation. Figure 40 is a normoblast with basophilic granules. Figure 45 is a cell containing both basophilic granules and nuclear particle.





normoblastic nucleus, or (2) the particles may arise as a result of direct fission, usually associated with nuclear condensation. Judging from the staining reactions, we conclude that the cells in which these changes were taking place were normal in every other respect. They usually stained like cells with the amount of hemoglobin of mature cells. Further, the nuclei of normoblasts, from which nuclear particles were seen to have arisen, and the nuclei of red cells, in which definite nuclear particles also occurred without evident division forms, were not decrepit and degenerating, but were, for the most part, to all appearances young, healthy nuclei. Occasionally, however, there were cells in which the normoblastic nuclei showed signs of degeneration, such as regular and irregular ring formation or intrabodily clumping of the protoplasm of fragmentation, together with a more acidophilic staining reaction with Wright's stain. These cells, as a rule, showed other degenerative changes. But with rare exception we have noted that the occurrence and divisions of the nuclear particles were not associated with degenerative cell changes.

Apparently direct fission occurred with equal regularity, no matter how small the particles became, and this seems to be the most common method by which the particles became smaller. No other definite scheme of division could be discovered.

There is reason to believe that many if not eventually all of the particles leave the blood cells by a process of extrusion, as shown in Figures 37, 38 and 39. This event may occur before the nuclear particles have reached their smallest size. We believe the finding of such figures shows this process is not an artefact in the preparations, because of the relative frequency in the finding and because of the number of recognizable, intensely staining nuclear bodies, entirely free from the cell bodies; and, further, that they were found in the thicker portions of the smears, where the least trauma would occur to the red cells in the making of the smears. These extruded nuclear particles were especially evident in the vital-stained preparations.

#### STAINING REACTIONS

As noted before, there was some discrepancy in the fixing and staining reactions of the bodies described by Howell, and this left some doubt as to their relation to true nuclear material. To clarify this point we made preparations by numerous ways. With Wright's stain the nuclear particles in our cases stained as intensely as nuclei. They were sharply defined, usually with a definite, smooth contour, and in no way were they different from small pycnotic nuclei. Jenner's stain gave equally sharp results.

After heat fixation for forty-five seconds at the spheroidal point for water, the bodies described were found to stain equally as well as the

nuclei with carbol-thionin or with methyl-green-pyronin and in undiminished numbers. After alcohol fixation and staining with Giemsa they were found to stain well. Alcohol and alcohol-formaldehyd and saturated mercury bichlorid preparations when stained with carbol-thionin were found to show the nuclear particles to stain as other nuclei.

The vital stains made with polychrome-methylene blue showed the bodies in the same relative frequency as when stained with Wright's stain.

Smears preserved for two months wrapped in paper showed the nuclear bodies as well as the fresh preparation when stained with Wright's stain. With Ehrlich-Pappenheim triacid stain after heat fixation, however, only part of the bodies stained distinctly, as nuclear bodies should, while others stained irregularly, or only with the faintest trace of green, especially in the periphery, and still others were unstained and appeared as round, clear refractile bodies in the substance of the red cell. Similar changes were noted with Ehrlich's hematoxylin and eosin, but with less frequency.

Preparations heated ten, twenty or thirty seconds at the spheroidal point for water and stained with carbol-thionin were not so good as those completely fixed at forty and forty-five seconds. Shorter heating apparently did not materially increase the intensity of the staining with the triacid stain.

Since heat-fixed preparations stained by other well-recognized nuclear stains gave good preparations, we do not hold the reaction with Ehrlich-Pappenheim triacid stain a valid argument against their being nuclear material, for this stain is well known to be somewhat less constant in its reaction than other blood stains, being especially sensitive to slight changes in the fixation procedure.

As a rule, the nuclear particles showed no signs of degeneration, such as changes in the staining reaction, clumping of nuclear substance, or vacuolization, unless the cell itself showed signs of degeneration.

Normoblasts (Figs. 42 and 45) were not infrequently found which showed not only normal nuclei, but definite nuclear particles, and in addition the so-called granules of basophilic degeneration, which stained red with methyl-green-pyronin. These granules were small and had no regular contour, whereas the nuclear particles were definite, concrete, smooth, round or oval bodies, no matter of what size. Cells (Fig. 28 and 29) were occasionally found with many small nuclear particles, which gave the impression that a nucleus had exploded. Owing to the size and definite character of these bodies, they were unquestionably nuclear particles of nuclear origin. It seems

clear to us, therefore, that the nuclear particles have no definite relation to the granules of basophilic degeneration.

While nuclear particles are most common after splenectomy, without corresponding increases in the granulations and nucleated red cells, there may be in other blood diseases, as pernicious anemia, marked granulations with increase in the number of nucleated red cells with none or very few nuclear particles.

#### COMMENT

There can be little doubt that a close relationship exists between the loss of splenic function and the appearance of large numbers of nuclear particles in the blood. We have shown, as has been observed by others, that the nuclear particles occur in large numbers within a few hours after the removal of the spleen, and they continue to be present after the blood has become in other respects normal. That they occur independent of a primary blood disease is shown by the fact that they occur after the spleen has been removed in normal animals and in men (traumatic rupture of spleen). In no other conditions are they found with such constancy and in such large numbers as after splenectomy.

Our studies have shown, further, that there is no definite numerical relationship between the nuclear particles and the presence of true nucleated red cells, or any other quantitative or qualitative changes in the peripheral blood. My preparations indicate that the nuclear particles originate from otherwise normal nuclei, and that the particles do not show in themselves qualitative degenerative processes.

It is true that following splenectomy there is evidence of increased bone marrow activity, as shown (1) by the increase in the number of nucleated red blood cells, (2) by the immediate increase in the polymorphonuclear neutrophils, and (3) by the increase in the large mononuclear and transitional forms. We include here the transitional and large mononuclear forms, because, as shown by Evans,<sup>23</sup> and to a less extent confirmed by me with the "indophenoblauf" reaction, these cells, in a large part at least, come from the bone marrow. These evidences of increased activity on the part of the bone marrow later subside, while the nuclear particles still persist.

Our staining reactions have shown these particles to be true nuclear material, and our drawings illustrate the way they arise from the nuclei of red blood cells; and since nuclear particles are present, due to the removal of the spleen, we conclude that the spleen in some way affects the normal disappearance of nuclear matter from the red blood cells. Such nuclear particles have been described in bone marrow, but

23. Evans: Observations on the Origin and Status of the So-Called Transitional White Blood Cells, *THE ARCHIVES INT. MED.*, 1916, **17**, 1.



to what extent apparently has not been determined. It is possible that we are dealing with an abnormal course of an otherwise normal process or that the process of denuclearization is arrested or slowed at some intermediary point, so that cells with the nuclear particles on the way to extrusion escape to the peripheral blood.

From the facts that they occur without any definite relationship to normoblastic crises, and persist regardless of the condition of the blood, whether after splenectomy in experimental animals, in man with or without blood diseases or in conditions of recovery after splenectomy in definite blood diseases, it would seem that they are not definitely associated with the process of regeneration, or at least cannot be taken as an index of regeneration.

It is possible that the loss of the splenic function so affects the ripening process of red cells that a more resistant cell is produced, and to this one might attribute in part at least the beneficial results of splenectomy.<sup>24</sup>

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24. In addition to the references already given, the following will be found of interest:

Moffit: Studies in Pernicious Anemia, Tr. Assn. Am. Phys., 1914.

Huber: Ueber den Einfluss der Milzextirpation bei perniziöser Anämie, Berl. klin. Wchnschr., 1913, **50**, 2179.

## THE EFFECT OF HEAT AND CONTINUOUS INCANDESCENT ELECTRIC LIGHT IN EXPERIMENTAL ARTHRITIS \*

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CHICAGO

In a previous paper<sup>1</sup> the writers found that the production of experimental arthritis was either prevented or was much milder in degree in rabbits treated with the continuous incandescent electric light than in those not so treated. Three of the five series however were in a cage the temperature of which was 15 to 20 C. above that of the room, giving a combined effect of heat and light. In one series the heat was eliminated to a great extent, but the series was too small to draw any definite conclusions. A comparison of the groups shows that of twelve rabbits treated with the light and heat there was an average of one lesion per animal, while the twelve controls had an average of 2.7 lesions per animal. In the series treated with light alone, the temperature being from 4 to 6 C. above the surrounding room temperature, six treated had an average of 1.7 lesions, six controls an average of 2.7 lesions. From this it would appear that light and heat produced the better results.

The following is a continuation of the study with some additional phases. Some of the animals were treated by heat alone, while others received as far as possible a simple light effect.

The rabbits used were about 1,000 gm. in weight. Those treated by means of an artificial temperature were placed after inoculation into a cage having glass sides and a metal door and top. This was heated from below by means of an electric stove or plate. Platforms were introduced within the cage, leaving air spaces between, in order to promote radiation in a uniform manner. The temperature was maintained at from 33 to 35 C.

The cage for light effects was of open wire, allowing a free circulation of air and containing three 150 watt Mazda globes. The temperature within this cage was from 4 to 6 C. above the room temperature.

The organism used was the *Streptococcus hemolyticus* of a sufficient dosage to produce arthritis in from 4 to 7 days, but not of such

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\* From the Department of Therapeutics and Experimental Medicine, University of Illinois, College of Medicine, Chicago.

1. THE ARCHIVES INT. MED., 1916, **17**, 78.

strength as to cause death. Injections were made into the marginal vein of the ear.

Six series of nine rabbits were used, including the controls. Three were placed in the box for heat treatment, three in the open cage for exposure to the effects of continuous incandescent electric light and three had no treatment.

In the first series the animals exposed to temperature effects presented one distinct lesion; those to the incandescent light, one; while the rabbits not treated had four joints affected.

The second group gave a result of no distinct lesions in the heat-treated, one in the light-exposed animals and two in those not treated.

The third series showed the heat-treated animals with no lesions, the light-treated with five and the untreated with no definite lesions.

As the controls were not having lesions, the dosage of the living streptococci for the following groups was increased.

The next results were three lesions in the heat-treated group, five in the light-treated, and fourteen in the untreated group. The first-mentioned were mild involvements, the second slightly more prominent, while the rabbits not under treatment showed eight severe joints, one animal being moribund when killed for necropsy.

The fifth group had the following involvements: heat-treated three, light-exposed seven, untreated thirteen lesions, six classified as very severe.

The last series gave the following result: animals in heat box, five; in light cage, nine; and untreated, seventeen arthritic lesions.

To review the above factors, heat-treated rabbits presented twelve distinct lesions, those exposed to light twenty-eight, and the animals not treated nearly twice as many, or 50 joints. One important feature was that the treated animals were recovering while some of the untreated were very sick when examined previous to necropsy. In other words, although the treatment did not prevent arthritis in all instances, the lesions were much milder and recovery more rapid, thus confirming our previous findings.<sup>1</sup>

The consideration of weights presents some interesting points, in that the heat-exposed rabbits gained only 559 gm., while those exposed to light gained 2,610 gm. The animals not under treatment gained 1,098 gm.

One might expect a higher body temperature in groups exposed to a higher degree of heat, but a composite average shows heat-exposed animals at 39.7 C. (103.4 F.), light-exposed, 39.8 C. (103.6 F.), and those at room temperature, 39.9 C. (103.8 F.).



As will be seen in Table 1, the number of joints involved per rabbit in the heat-treated group was 0.7, or a total of twelve, one of these classed as severe; the number of joint lesions in the light-treated was 1.6, or a total of twenty-eight, seven being severe; and the number per rabbit in the untreated was 2.7 or a total of fifty, nineteen being severe.

There were five deaths among the heat-treated rabbits, while none occurred in the light-treated or the untreated. Postmortem examinations demonstrated pulmonary lesions in each instance as the cause of death.

The animals treated by heat did not gain as well as the light-treated rabbits and were more prone to pulmonary affections. The light treated animals, while they exhibited a higher average of lesions, gained more weight and death did not occur in any of the rabbits so treated. The untreated rabbits gained less than the light-treated and seemed in greater distress than either of the other series, their lesions were more numerous and severe, but did not result in fatalities.

TABLE 1.—COMPARISON OF THE TOTAL RESULTS IN EIGHTEEN RABBITS TREATED BY HEAT, EIGHTEEN BY LIGHT AND HEAT, WITH A SIMILAR NUMBER OF UNTREATED ANIMALS

	Heat Treated	Light Treated	Untreated
Number of joints involved per rabbit.....	0.7	1.6	2.7
Severe lesions .....	1	7	19
Average gain in grams.....	31	145	61
Average daily temperature .....	103.4	103.6	103.8
Average maximum temperature .....	105.6	105.2	105.4
Deaths .....	5	0	0

Further experiments now in progress to ascertain the effect of continuous light with heat produced by the light, and the effect of light and heat in treating lesions after their appearance, further tend to confirm our observations. In fact the results have been so encouraging that apparatus is being constructed for the treatment of human patients.

#### CONCLUSION

It is evident from the results of our experiments that the treated animals were decidedly benefited as far as the arthritic manifestations were concerned. We feel that our series is too small as yet, and because the deaths from pulmonary infection somewhat vitiated its results we cannot draw any definite conclusions regarding the merits of dark heat versus light.

## FURTHER QUANTITATIVE STUDY OF THE DUODENAL BLOOD-DERIVED PIGMENTS \*

J. P. SCHNEIDER, M.D.

MINNEAPOLIS

In this, our second year's work, we have entertained with reference particularly to pernicious anemia a twofold objective, namely, the determination of pigment values after splenectomy in cases estimated quantitatively prior to operative interferences, and a critical study of the relationship between the pigment output and red cell count in parenteral anemias. It would seem that a study of the blood-derived pigments before and after splenectomy as undertaken above might shed some light on the reasonableness of the hypothesis of hypersplenism; whereas the latter analyses would serve to demonstrate indirectly the state of the bone marrow. Incidentally a variety of non-hemolytic diseases have been studied, including enteral-bleeding anemias with pernicious-like blood pictures.

Following precisely the technic elaborated in our previous work<sup>1</sup> we made a total of fifty-seven determinations in forty individual patients, which are here recorded. Of these, twenty-one are cases of pernicious anemia and one hemolytic icterus. A total of seven patients with pernicious anemia of this series and one with hemolytic icterus have been splenectomized. Three of the former have been studied both before and after the operation. Two patients are included in whom splenectomy had been performed a year previous.<sup>2</sup> The material for this study has been drawn largely from the University Hospital and outpatient clinic, ten are referred patients and six were studied at the Rochester Clinic.

In Table 1<sup>3</sup> are grouped together the determinations made in cases of pernicious anemia and hemolytic icterus with spleens retained. A

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\* Read at the April, 1916, meeting of the Minnesota Pathological Society.

1. Schneider, J. P.: The Splenic Pathology of Pernicious Anemia and Allied Conditions, *THE ARCHIVES INT. MED.*, 1916, **17**, 32.

2. In no instance is a determination included in which the slightest variation in technic obtained, or such as not strictly fresh duodenal contents were used. The Kirchoff and Bunsen large model spectroscope was used in all of these determinations, diluting the original contents as indicated in our previous paper. A handy-sized instrument, so constructed as to lend itself to a reading of the values of urobilin and urobilinogen on a micrometer scale without the necessity of diluting the original solution, is in the course of construction.

3. The term "H.-H. Index" heading the last column is an abbreviation for the proposed designation, "hematopoietic-hemolytic index," the nature and meaning of which will appear subsequently in the text.

mere glance at the table will serve to show, as we demonstrated in our last year's work, the striking values of urobilin and urobilinogen in the hemolytic anemias. Urobilinogen, which is normally absent or present as a mere trace, may and at times does exceed in value that of urobilin. In no undoubted case of pernicious anemia has the total value of the two pigments been less than 2,000.

TABLE 1.—DETERMINATIONS IN CASES OF PERNICIOUS ANEMIA WITH SPLEENS RETAINED

Case No.	Date	Name	Blood Count	Hemo-globin	Index	Bilirubin	Urobilin	Urobilin-ogen	Total	H.-H. Index
1	5/24/15	J. A.	2,800,000	70	1.3	+++	1,400	1,600	3,000	0.97
1	6/ 3/15	J. A.	3,200,000	62	1 —	+++	1,200	1,400	2,800	1
1	7/22/15	J. A.	4,000,000	65	0.8	+++	1,400	1,200	2,600	1.1
2	5/ 3/15	Mr. S.	2,000,000	37	0.92	+++	1,800	2,000	3,800	0.97
2	6/22/15	Mr. S.	Not recorded	..	....	+++	2,000	3,600	5,600	....
2	6/22/15	Mr. S.	3,600,000	60	0.83	+++	1,400	1,600	3,000	1.1
3	6/10/15	Mr. B.	1,700,000	30	0.9	+++	2,200	3,200	5,400	1.1
3	10/23/15	Mr. B.	1,000,000	25	1.2	+++	2,000	2,200	4,200	0.86
4	9/16/15	Mrs. B.	2,500,000	45	0.9	+++	1,400	1,200	2,600	0.85
5	10/ 6/15	C. S.	1,300,000	36	1.4	+++	1,400	1,000	2,400	0.61
6	3/ 1/16	Mrs. T.	1,600,000	36	1.1	+++	2,200	600	2,800	0.73
7	3/ 2/16	Miss G.*	2,840,000	47	0.8	+++	4,600	1,000	5,600	1.4
8	3/ 2/16	W. H.	1,960,000	38	0.9	+++	2,000	600	2,600	0.75
9	3/ 3/16	Miss L.	2,940,000	50	0.8	+++	5,000	1,000	6,000	1.4
10	3/14/16	Mrs. S.	4,000,000	78	0.98	+++	1,000	1,000	2,000	1
11	3/16/16	Mr. P.	2,000,000	48	1.2	+++	2,400	800	3,200	0.87
12	3/19/16	W. M.	1,500,000	31	1 *	+++	1,600	600	2,200	0.61
13	3/22/16	Mr. M.	2,500,000	48	0.9	+++	1,800	800	2,600	0.85
14	3/28/16	Mr. H.	1,300,000	20	0.8—	+++	2,800	800	3,600	0.81
15	4/10/16	Mrs. S.	1,500,000	25	0.83	+++	3,600	2,000	5,600	1.1
16	6/14/16	Mrs. F.	2,400,000	43	0.9	+++	1,400	1,600	3,000	0.9
17	5/20/16	J. B.	1,750,000	35	1	+++	1,600	800	2,400	0.7
18	3/12/16	Dr. M.	1,500,000	32	1	+++	1,400	600	2,000	0.6
19	4/20/16	Mr. H.	1,270,000	20	0.8	+++	2,400	800	3,200	0.73
20	5/20/16	Mrs. H.	1,970,000	30	0.8	++	1,200	400	1,600	0.6

\* A case of hemolytic icterus.

Case 38 would appear to be an exception; but while it is listed as a case of pernicious anemia, there are several other features not in accord with the findings generally observed, namely, the lack of pleochromia in the bilirubin values and the physical findings of emaciation, certainly rare in pernicious anemia. Case 1 is a continuation study of a last year's patient, dealt with fully in Curve 1.



Table 2 embraces five of the seven cases of splenectomized patients. Of these, three were observed over a long period of time, repeated duodenal estimations having been made after the operation in order to establish the nonaccidental nature of the findings. It will be observed that the pigment values suffer a definite reduction to a normal level as soon as two weeks after ablation of the spleen and remain at that level as long as studied, in Case 5 during the ensuing five

TABLE 2.—DETERMINATIONS IN FIVE CASES OF PERNICIOUS ANEMIA BEFORE AND AFTER SPLENECTOMY

Case No.	Date	Name	Blood Count	Hemo-globin	Index	Bilirubin	Urobilin	Urobilin-ogen	Total	H.-H. Index
Before splenectomy 4	9/16/15	Mrs. B.	2,500,000	45	0.9	+++	1,400	1,200	2,600	0.85
After splenectomy 4	11/16/15	Mrs. B.	3,900,000	70	0.9	++	400	0	400	
4	12/22/15	Mrs. B.	3,500,000	65	0.92	++	1,200	200	1,400	
4	2/23/16	Mrs. B.	4,300,000	71	0.92	++	1,200	0	1,200	
Before splenectomy 5	10/ 6/15	C. S.	1,300,000	36	1.4	+++	1,400	1,000	2,400	0.61
After splenectomy 5	11/ 1/15	O. S.	1,900,000	30	0.8	++	1,000	200	1,200	
5	11/26/15	C. S.	3,000,000	70	1.3	++	1,000	0	1,000	
5	12/ 8/15	C. S.	4,000,000	70	0.87	++	800	0	800	
5	1/14/16	C. S.	3,500,000	70	1	++	1,400	0	1,400	
5	3/ 7/16	C. S.	4,700,000	83	1	++	800	0	800	
Before splenectomy 15	4/10/16	Mrs. S.	1,500,000	25	0.83	+++	3,600	2,000	5,600	1.1
After splenectomy 15	4/30/16	Mrs. S.	2,500,000	39	0.8	++	1,200	200	1,400	
After splenectomy 21	3/ 2/16	M. S.	*	..	.....	++	400	0	400	
22	5/ 1/16	Mr. S.	1,750,000	Not recorded	.....	++	1,200	2,000	3,400	

\* Not recorded but moderately low count.

months. Ten months have elapsed since patients in Cases 4 and 5 were splenectomized, five since the patient in Case 15 was operated on. All are in good health with the exception of a persistence of the cord changes.

That a reestablishment of pathologic hemolysis with the resulting increased blood-derived pigments does not occur in postsplenectomized cases when a recurrence apparently sets in a year later is strongly probable from the findings in Cases 21 and 22 of Table 2. The patient

in Case 21 had been splenectomized, a brilliant postoperative blood rise recorded, apparently a complete return to good health established, when under the stress of business cares a recurrence took place. A duodenal estimation at this time records nonhemolytic values—on the contrary, oligochromemia. The most plausible explanation would be that the hematopoietic function was below normal delivery at the time of the operative interference and subsequently failed more and more

TABLE 3.—DETERMINATIONS IN VARIOUS DISEASES

Case No.	Date	Name	Disease	Blood Count	Hemoglobin	Index	Billirubin	Urobilin	Urobilinogen	Total
1	4/26/15	P. W.	Psychosis.....	5,000,000	100	1	++	900	0	900
1	4/29/15	P. W.	Psychosis.....	5,000,000	100	1	++	1,000	0	1,000
2	4/26/15	Mrs. W.	Pericholecystitis.....	4,200,000	85	1	+	800	0	800
3	5/31/15	Mrs. H.	Liver hydatid.....	4,600,000	90	1	++	600	0	600
4	5/16/15	Mrs. S.	Secondary anemia.....	2,800,000	60	1	++	200	0	200
5	2/ 1/16	Mr. R.	Infectious icterus.....	4,600,000	90	1	+	200	0	200
6	11/ 3/15	Mr. M.	Hemochromatosis.....	4,800,000	75	0.78	++	1,400	0	1,400
7	2/ 7/16	Mr. O.	Gastric carcinoma*.....	3,100,000	50	0.8	+	400	0	400
8	2/12/16	J. H.	Lymphatic leukemia....	4,000,000	70	0.87	+	1,400	0	1,400
9	3/ 1/16	M. K.	Advanced cirrhosis.....	3,530,000	70	1	+	1,200	Trace	1,200
10	3/10/16	Mrs. M.	Doubtful.....	3,500,000	85	1.2	++	1,000	0	1,000
11	3/11/16	Mrs. D.	Gastric carcinoma.....	1,200,000	20	0.8	+	200	0	200
12	3/12/16	C. D.	Duodenal ulcer.....	3,600,000	40	0.55	+	600	0	600
13	3/14/16	Mr. W.	Gastric carcinoma.....	1,250,000	25	1	+	200	0	200
14	8/ 2/16	Mrs. S.	Gastric carcinoma.....	2,640,000	35	0.7	++	600	0	600
15	6/23/16	Mr. O.	Stone obstruction.....	.....	...	...	+	200	0	200
16	6/22/16	Prof. S.	Neurosis.....	4,800,000	80	0.9	++	1,200	0	1,200
17	6/13/16	O. H.	Unknown.....	4,200,000	69	0.8	+	200	Trace	200
18	4/20/16	Mr. C. C.	Gastric carcinoma*.....	2,000,000	40	1	+	400	0	400
19	5/16/16	Mrs. C.	Secondary anemia.....	2,970,000	60	1	++	1,200	Trace	1,200
20	5/29/16	Mrs. C.	Secondary anemia.....	2,850,000	60	1	++	1,000	Trace	1,000
21	5/25/16	Mr. O.	Acute catarrhal icterus†	4,800,000	90	0.9	+	1,200	0	1,200

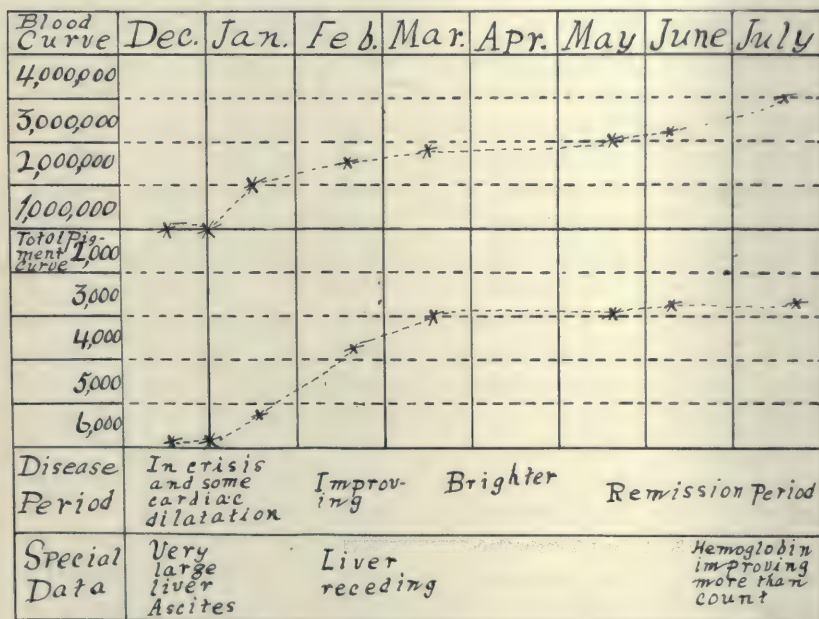
\* Confirmed at operation (guaiac positive in duodenal contents).

† Urine estimated at the same time gave urobilin 1,000, urobilinogen 600 and billirubin ++.

under demand. The patient in Case 22 was seen at a similar period postoperative and had had the rather usual early betterment only to experience a profound recurrence, in which he was seen and studied. To our amazement the duodenal contents looked dark and yielded the extremely high values recorded. However, a quantitative urine estimation demonstrated what I anticipated, even higher values. This clarified the atmosphere. An extremely dilated heart, with fourth degree incompensation, and a consequent severe hepatic stasis accounted for

the findings; for it must be remarked that it is always well to guard against this possible error by noting the state of the circulation and estimating in doubtful instances the quantitative urinary pigments.

From the facts gathered together in Table 2 we can proceed with the assumption that splenectomy ablates one factor in the equation  $x-y=z$ , namely,  $y$ . However, varying with type and stage, this factor may be the lesser of the two. To estimate with some degree of accuracy this second factor,  $x$ , would mean the ability to judge in advance the probable effectiveness of splenectomy.



Curve 1.—Total red count and pigment values encountered in Case 1, portraying the remarkable parallelism between the two.

From a study of Curve 1, representing graphic parallel curves of the blood and pigment values encountered in a case under observation and study for a period of seven months, from the normal pigment level possible of designation from the data here gathered together in disease not affecting the blood, and from a study of the comparative blood and pigment values found in hemolytic icterus, in which splenectomy is so strikingly efficient, we are approaching the solution of factor  $x$ , in the above equation  $x-y=z$ , in which  $x$  represents hematopoiesis,  $y$  splenism<sup>4</sup> and  $z$  the resulting blood count. Normally the total pigments range around 1,000. This then represents physiologic splenism. The blood count stands around 5,000,000. Given the value therefor of  $y$  and  $z$ ,  $x$  can readily be determined. Reduced to simple com-

4. Spleen-liver complex.



parative figures:  $x - 1 = 5$ ; that is,  $x = 6$ . In a given unit of time 6 parts are supplied, one physiologically sacrificed, leaving a constant normal of 5. With  $x$  remaining constant, we can tabulate as follows: ( $x - y = z$ );  $6 - 1 = 5$ ;  $6 - 2 = 4$ ;  $6 - 3 = 3$ ;  $6 - 4 = 2$ ;  $6 - 5 = 1$ .

Now from Curve 1 we gather the information that as the blood count rises the pigment values fall. This makes it self-evident that the other possible table of variations cannot obtain in pernicious anemia; to wit: ( $x - y = z$ );  $6 - 1 = 5$ ;  $5 - 1 = 4$ ;  $4 - 1 = 3$ ;  $3 - 1 = 2$ ;  $2 - 1 = 1$ .

For in this relationship the value of  $y$  is constant and low.

To determine, hence, in a given case what may be styled the hematopoietic-hemolytic index resort may be had to the formula, H.-H. Index  $= \frac{z+y}{x}$ , in which  $y$  represents the first figure of the blood count,  $z$  the same of the pigment values in round numbers, and the value of  $x$  is 6. In Case 11, Table 1, for instance, the blood count standing at 2,000,000, the pigment values at 3,200, the hematopoietic-hemolytic index is  $\frac{2+3.2}{6} = \frac{5.2}{6}$  or 0.87. With the index less than normal by a slight margin only, a marrow possible of recovery to a normal output might be hoped for. While, on the contrary, in a case of the type of Case 12, with the blood at 1,500,000 and the pigment at 2,200, the index  $= \frac{1.5+2.2}{6} = \frac{3.7}{6} = 0.6$ —a severely negative index.—hypohematopoiesis—and a restitution ad integrum would seem improbable. Contrasting these indexes in the slowly recovering pernicious anemias after splenectomy with the index in hemolytic icterus, as illustrated in Case 7, in which the blood picture approaches the normal in a short space of time, we find that with the blood count at 2,500,000 and the pigment total at 5,600 the index is heavily plus, namely, 1.4.

Judged by the hemolytic index, Cases 1, 2, 3, 4, 9, 10, 11, 15 and 16 appear favorable for splenectomy. Case 11 bears this out in two clinical features, the persisting icterus, pointed out as a favorable indication in our former paper, and a definitely enlarged, hardened spleen. Early in the disease, not necessarily in the mere point of time, this organ is definitely enlarged in favorable types.

In the face of the fact that urobilinogen is peculiarly and intimately associated with structural changes in the total liver parenchyma, it will be reserved for future confirmation whether for the index the total of both urobilinogen and urobilin be the proper value of  $y$  or urobilin only.

Incidentally, this study of duodenal pigment values has served to provide a most simple and reliable method of differentiating severe anemias due to enteric bleeding from parenteral types. Cases 7, 13,

14 and 18 in Table 3 were regarded as parenteral in type. In Case 7 with the absence roentgenographically of a persistent filling defect and of motor disturbances, of lactic acid and the related organism, and of occult blood in the stomach contents, with a blood picture so typical of the pernicious type as to be tentatively so regarded for months, it remained for the duodenal pigment analysis to definitely decide the case to be one of enteric bleeding. In Case 13 the imitation was complete, even to the point of typical remission periods. Here, after repeated trials, a positive guaiac in the stools was of help.

The more pronounced the degree of anemia, and hence the closer the blood picture imitation of the parenteral type, the more strikingly are the pigment values at the opposite pole of oligochromemia in enteric bleeding.

#### SUMMARY

1. Splenectomy apparently immediately and permanently reduces the excessive blood-derived pigments of pathologic hemolysis to a normal level. There is no proof that a recurrence of a pernicious blood and clinical picture after splenectomy is due to a reestablishment of excessive blood destruction.

2. Bearing in mind that fully developed pernicious anemia is a late bone-marrow exhaustion of a primary hemolytic process, we know our endeavor to gain a preoperative knowledge of the competency of the marrow is necessary to a proper selection of favorable operative cases. A plus hematopoietic-hemolytic index in a given case should be regarded as one factor favorable to permanent postoperative recovery.

3. The classic blood picture of pernicious anemia may be successfully imitated by chronic enteric bleeding anemia. For a reliable means of differentiating these fundamentally dissimilar conditions, a quantitative estimation of the duodenal pigments is decisive.

Our thanks are extended to the Rochester Clinic and Dr. Tuohy of Duluth for the opportunity graciously accorded us of making the above studies on private patients.

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## OSTEOGENESIS IMPERFECTA \*

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### DEFINITION

Osteogenesis imperfecta is a rare systemic disease of unknown etiology, characterized by imperfect development of bones. Increased absorption of deficiently formed bony trabeculae leads to deficient formation of cortex and spongiosa, which results in osteoporosis. Clinically it is characterized by multiple intraperiosteal fractures, usually with little displacement of fragments and excessive callus formation. It occurs in two clinical forms, namely, osteogenesis imperfecta congenita and osteogenesis imperfecta tarda.

### SYNONYMS

The name osteogenesis imperfecta congenita was coined by Vrolik<sup>1</sup> in 1845. Although a number of other names have been proposed for this disease, Vrolik's designation seems to be the best, since it is derived from pathology of the disease and does not assume or presuppose anything. Synonyms for this disease are as follows: osteomalacia congenita, because in some cases the bones are abnormally pliable; rachitis foetalis annularis, because of the excessive callus formation at the seat of fractures; malacia myeloplastica (von Recklinghausen<sup>2</sup>), periosteal dysplasia (Durante<sup>3</sup>), dystrophie periostale (France), ostitis parenchymatosa chronica (Schmidt<sup>4</sup>), micromelia annularis, osteoporosis congenita (Kundrat), fragilitas ossium (Klebs), from the chief symptom, which is fragility of the bones. Fragility of the bones, however, is present also in other diseases, among others rachitis, osteomalacia, scurvy, tuberculosis, hereditary syphilis and miscellaneous neoplasms. In these cases, however, it is secondary, while in

\* Submitted for publication July 18, 1916.

1. Vrolik: Tabul. ad illustrandam embryogenesin. hominis et mammalium, Amstelodami, 1845, Tab. 91.

2. Von Recklinghausen: Untersuchungen über Rachitis und Osteomalazie, Fischer, Jena, 1910.

3. Durante: La dysplasie périostale, Acad. de méd., 1905.

4. Schmidt: Ein Beitrag zur Kenntnis der sogenannten Osteopsathyrosis congenita, Inaug.-Dis., Leipzig, 1901; Abnorme Knochenbrüchigkeit bei einem Neugeborenen, Monatschr. f. Geburtsh. u. Gynäk., 14.



osteogenesis imperfecta congenita the fragility of the bones is a primary manifestation.

Osteopsathyrosis idiopathica is a name which was given by Lobstein<sup>5</sup> in 1833 to a disease which in its pathology and symptoms resembles the osteogenesis imperfecta; however, he described it as occurring in older infants and children. Today these two diseases are regarded as identical processes, Lobstein's description corresponding to osteogenesis imperfecta tarda. Looser<sup>6</sup> also thinks that "the so-called idiopathic osteopsathyrosis and the osteogenesis imperfecta are pathologically identical diseases. As to the clinical onset of the disease, all imaginable transitions are present between the congenital affection and one appearing only in later infancy or childhood. Even those cases of osteogenesis imperfecta (in broader sense) that make their appearance in childhood are probably to be traced back to congenital changes. Durante<sup>3</sup> says that osteopsathyrosis of the adult represents an atypical form of osteogenesis imperfecta. Miura<sup>7</sup> speaks of a secondary osteopsathyrosis which occurs in childhood and is due to rickets, osteomalacia, Barlow's disease, tuberculosis, hereditary syphilis and miscellaneous neoplasms, in contradistinction to a primary fragility of bones, so-called osteopsathyrosis idiopathica, the pathogenesis of which is not well understood yet. It occurs in two types: The one is congenital and is identified with osteogenesis imperfecta by most authors; the other occurs in early childhood. Summing up the views of different authors as to the relation between osteogenesis imperfecta congenita and osteopsathyrosis idiopathica, Frangenheim<sup>8</sup> says:

With Looser we are of the opinion that the osteogenesis imperfecta and the idiopathic osteopsathyrosis represent a form of a disease which, as far as histologic behavior of the bones is concerned, has nothing to do with any known disease accompanied by atrophy or softening of the skeleton, the most important symptom of the disease being the abnormal fragility of the bones, which appears at birth or very soon after birth, but may also appear in early or late childhood after a certain period of latency. If we want to separate the osteogenesis imperfecta from the idiopathic osteopsathyrosis, then the difference can be only a clinical one, depending on the time at which the fragility of the bones begins.

According to this there are two forms of osteogenesis imperfecta, one which is congenital (congenita), the symptoms appearing at birth or very soon after birth, and the other a latent form (tarda), in which the symptoms appear in late infancy or childhood, after a variable

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5. Lobstein: *Lehrb. d. pathol. Anat.*, 1834, **2**, 179.

6. Looser: *Zur Kenntnis der Osteogenesis imperfecta congenita und tarda*, *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1905, **15**, 161. *Ueber Osteogenesis imperfecta tarda*, *Verhandl. d. deutsch. path. Gesellsch.*, 1905, p. 239.

7. Miura, S.: *Osteopsathyrosis Idiopathica*, *Jahrb. f. Kinderh.*, 1911, **73**, 545.

8. Frangenheim: *Die Krankheiten des Knochensystems im Kindesalter*, *Neue Deutsche Chirurgie*, Enke, Stuttgart, 1913, **10**.

period of latency, the latter being sometimes called osteopsathyrosis idiopathica. In this respect the disease resembles congenital syphilis, in which the manifestations may appear at birth or very soon after birth, or they may appear in late infancy or childhood, after a variable period of latency.

#### ETIOLOGY

The direct cause of the disease is not known. There is some evidence which seems to show that the disease may be inherited. Griffith<sup>9</sup> estimated a hereditary factor in 27 per cent. of all cases of osteogenesis imperfecta congenita and in 15 per cent. of cases of osteogenesis imperfecta tarda, these figures being in all probability far too low. Harmer says that it has been transmitted through three generations and it has affected nine of ten children and a child of one of these. Zurhelle<sup>10</sup> reports a case of a child in whose mother he found a deformity of a leg and the Roentgen rays showed healed fractures and osteoporosis. Schmidt<sup>11</sup> mentions a case of a 68-year-old man who suffered many fractures before the 20th year of life, all of them being due to trivial causes. He had two sons. One had fractures at birth and the other suffered numerous fractures in the first year of his life. Gurlt<sup>12</sup> compares the disease with hemophilia, which shows the same territorial distribution, namely, North Germany, England and North America, and states that it is transmitted to the males through unaffected females. Looser and Griffith, however, say that the disease is seldom transmitted through female offspring. Several generations may be affected and one generation may be skipped. The congenital form occurs more frequently in females (Frangenheim<sup>8</sup>), the late form more often in males. Calcium deficiency was suspected to be the cause of the disease and Stoelzner was able to produce changes identical with osteogenesis imperfecta in animals by feeding them with food poor in calcium. Looser, however, remarks that it is very difficult to administer calcium-free food to man, and besides, children suffering with osteogenesis imperfecta received the same food as their parents and brothers and sisters who did not suffer. Prematurity was suggested as a cause also. Syphilis was also suspected; both parents were syphilitic in Vrolik's case and the mother was syphilitic in Henckel's<sup>13</sup> case, but in neither case did the children show

9. Griffith: Idiopathic Osteopsathyrosis (Fragillitas Ossium) in Infancy and Childhood, *Am. Jour. Med. Sc.*, 1897, **113**.

10. Zurhelle: Osteogenesis imperfecta bei Mutter und Kind, *Beitrag zur Frage der Identität dieser Erkrankung mit Osteopsathyrosis idiopathica*, *Ztschr. f. Geburtsh. u. Gynäk.*, 1913, **74**, 942.

11. Schmidt: Demonstration von Röntgenbildern eines achtjährigen Knaben mit Osteopsathyrosis, *München. med. Wchnschr.*, 1899, No. 22, p. 748.

12. Gurlt: Ueber Knochenbrüchigkeit un über Frakturen durch Muskelaktion, *Deutsch. Klin.*, 1857; *Handbook of Bone Fractures*, 1862, **1**.

13. Henckel: *Neue medizinische und chirurgische Anmerkungen*, Berlin, 1772.



any symptoms of syphilis. Glands of internal secretion, especially thyroid and hypophysis, have been searched for the change which may have caused the disease, but no marked changes have been described in these organs.

#### PATHOGENESIS

The disease consists of imperfect formation of bones. The development of bones is normal up to formation of primary marrow spaces. The spaces are not lined by bony cells, as they should be, and this starts the abnormal development. All observers agree that there is deficient formation of bone, but there are some differences of opinion as to whether the process of absorption of bone, which is a normal process during the growth, remains within normal limits. Thus Axhausen<sup>14</sup> says that the nature of the disease is a deficient enchondral and periosteal bone formation, due to lowered function of osteoblasts and of the periosteal cells, while the preparatory calcification of cartilage and absorption of bone are normal. Segawa,<sup>15</sup> on the other hand, says that the pathologic process in the bone in osteogenesis imperfecta congenita consists of a disproportion between building up and breaking down, which leads to porosis, deficient apposition or increased osteoclastic resorption playing important rôles, individually or combined. These deviations from the normal result in (1) absence of successive thickening of corticalis toward the middle of the diaphysis; (2) the bone formed from the periosteum remains in the primitive stage of fibrous bone; (3) the reticular structure shows wide meshes, the Haversian canals being replaced by large marrow spaces; (4) the osteoporosis in all probability is secondary to excessive formation of the medullary spaces, which again is due to deficient development of the bony substance and to increased absorption. The more remote cause of this maldevelopment is supposed to be embryonal malformation of periosteum and endosteum at the beginning of the process of ossification (Dieterle) or to the circumstance that the osteoblasts that arise from avascular, fibrous bone marrow because of insufficient nutrition do not develop properly, remaining polygonal and low, and later many of them undergoing metaplasia into osteoclasts (Buday<sup>16</sup>). An objection, however, might be made to the latter theory, since avascular, fibrous marrow is not found in all cases.

14. Axhausen: Osteogenesis imperfecta oder frühe Osteomalazie als Grundlage der idiopathischen Osteopsathyrosis, *Deutsch. Ztschr. f. Chir.*, 1908, **92**, Nos. 1-3; Zur Frage der Osteomalazie im Kindesalter, *Gedenkschrift f. v. Leutbold*, 1906, **2**.

15. Segawa: Combination of Congenital and Acquired Diseases of the Skeleton (Osteogenesis Imperfecta Congenita, Morbus Barlowii, Rachitis), *Ztschr. f. Kinderh.*, 1915, **12**, 246.

16. Buday: Beitrag zur Lehre von der Osteogenesis imperfecta, *Sitzungsberichte d. k. Akademie zu Wien. Math.-naturwissensch. Klasse*, 1895, **104**, Nos. 1-5.



## PATHOLOGY

The primary pathologic changes are of the same nature in all bones of the body, varying only in degree. These changes are best observed in the bones of the extremities. The bones are either so soft that they may easily be bent or cut with a knife or they are brittle. They may be plump and short or compressed or cylindrical. The shortening is secondary to fractures and bendings, the primary growth in length being normal. Numerous thickenings due to healed fractures may be seen. The convexity of the deformities in the bones are on the extensor surfaces. Bluish white islands of cartilage are seen throughout the spongiosa. Epiphyses are formed by hard hyaline cartilage, which is surrounded by perichondrium. They are richly vascular and the centers of ossification are large. The epiphyseal line is straight. The proliferation zone of cartilage is narrower and somewhat irregular, the cartilage cells being arranged into columns that are about one fourth of the normal height. This is more marked toward the periphery. The calcified cartilage disappears too early, being prematurely absorbed. The bony trabeculae are short and thin and are placed obliquely and transversely to the long axis of the bone, while normally they are more longitudinal. There is a marked preponderance of medullary substance over spongiosa and the lamellar growth of the bone is less marked. The bone marrow is mostly fibrous, poor in cells and surrounded by bony trabeculae. Lymph marrow preponderates in the middle of the diaphyses (Buday<sup>16</sup>). Michel<sup>17</sup> found the marrow to be vascular only in the periphery of the bone and avascular at the epiphyseal line and where the bone is bent. Harbitz<sup>18</sup> described the bone marrow as very vascular and rich in cells, while Stilling<sup>19</sup> says that it is of normal vascularity. Osteoblasts are everywhere markedly diminished in number, but they are somewhat larger and their nuclei are broad and oval. Osteoclasts are strikingly too numerous.

## SYMPTOMS

Multiple fractures occurring on very slight provocation are the most prominent characteristic of the disease. Fractures are mostly complete with preserved periosteum, this being regarded by Segawa<sup>15</sup> as of importance for diagnosis. In recent fractures the fragments are connected by fibrous tissue, in older ones by spongiosa. The callus that forms in place of fractures is usually excessive in size, but it is of

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17. Michel: *Osteogenesis imperfecta*, Virchows Arch. f. path. Anat., 1903, 173.

18. Harbitz: *Ueber Osteogenesis imperfecta*, Beitr. z. path. Anat. u. z. allg. Path., 1901, p. 30.

19. Stilling: *Osteogenesis imperfecta*, Virchows Arch. f. path. Anat., 1889, 115, 3.

inferior quality histologically, being porous. The periosteum of the callus is broadened and rich in cells. Bone marrow is abundant, being gelatinous or finely fibrillar. Periosteal callus preponderates over the myelogenous. The callus is laid down in cartilage first, which is followed by metaplasia of cartilage into bone. When the callus is fully developed it consists of a network of calcified bony trabeculae with osteoblastic covering, with islands of hyaline cartilage below the periosteum and irregular arrangement of bony lamellae. The size of the callus depends mainly on the development of germinal and cartilage tissue, while the bone tissue is just as deficient as in other parts of the skeleton (Segawa<sup>15</sup>).

The so-called calvaria membranacea is also characteristic of the disease. The cranial vault resembles a soft rubber sac with mosaic-like inlays of small pieces of bones around the centers of ossification. The fontanels are widely open and abnormally large. The bones may be so brittle that on pressure they easily crumble to pieces. The membranous sac is formed by connective tissue membrane, which is poor in cells. The diploe is not formed. These findings apply especially to osteogenesis imperfecta congenita.

#### METABOLISM

Schwarz and Bass,<sup>20</sup> who have studied metabolism in a case of osteogenesis imperfecta congenita, found that nitrogen metabolism is approximately normal, fat retention and resorption is normal. Calcium retention was only slightly below the normal, there being, however, a positive calcium balance. In two cases of osteogenesis imperfecta congenita Bookman<sup>21</sup> found that during the active stage phosphorus retention was materially higher than that of two normal children examined by Cronheim and Müller;<sup>22</sup> magnesium retention was low during the active stage, but this increased with the increase in calcium retention during the healing stage, the increase in magnesium retention, however, being less marked than in calcium. As to calcium metabolism, Bookman came to the following conclusions, based on the study of two cases of osteogenesis imperfecta congenita, age approximately 3 and 4 months: 1. In active cases the calcium retention is somewhat below or very decidedly below the normal. 2. It is probable that variations in the course of the disease cause changes in the calcium balance. 3. The deficient retention of calcium is apparently influenced favorably by cod liver oil and phosphorus, and still more strongly by calcium lactate.

20. Schwarz and Bass: Osteogenesis Imperfecta: Report of a Case with the Study of Its Metabolism, *Am. Jour. Dis. Child.*, 1913, **5**, 131.

21. Bookman, A.: The Metabolism in Osteogenesis Imperfecta with Special Reference to Calcium, *Am. Jour. Dis. Child.*, 1914, **7**, 436.

22. Cronheim and Müller: *Biochem. Ztschr.*, 1908, **9**, 76.

## GENERAL SYMPTOMS

The children suffering with this disease are usually mentally underdeveloped, are small and underweight for their age and their skin is soft and delicate. Segawa observed in his case attacks of long-lasting profuse sweating. These attacks started in the second week of life, sweating being especially marked on the head and in the face and this lasted with remissions and recurrences up to death. The head of these children is usually excessive in size in proportion to the trunk and often covered with luxuriant, long, silky hair. The face is small and well developed, palpebral fissure narrow, but the physiognomy is not characteristic. The neck is short, the thorax is flattened, asymmetric, and the ribs show nodules which are calluses remaining after the healing of fractures. In lateral regions of the thorax deep retractions can be seen during breathing and crying. The skin of the thorax is delicate and edematous, subcutaneous tissue being richly developed. From the axillae deep folds extend over the chest. The abdomen is protuberant. The extremities are shortened, curved, angulated, showing calluses of fractures. The feet and hands are small and delicate. Flaccid paralysis of extremities, lowered electrical excitability and nystagmus have been found (Segawa<sup>15</sup>). A majority of children affected with this disease die soon after birth or are stillborn. In those surviving, the fractures may become less frequent with advancing age and the disease may come to a standstill.

The most characteristic symptom of the disease is multiple fractures, as many as 113 having been found by Chaussier.<sup>23</sup> The fractures may occur in series with periods between, during which the patient may be free from fractures. There is little pain associated with them and crepitus is often absent. Healing is more rapid than in normal bone, but marked deformity usually results. It is interesting to note that while fractures heal rapidly, osteotomies that have been undertaken for correction of deformities healed very slowly (Cortes,<sup>24</sup> Doering<sup>25</sup>).

## DIAGNOSIS

Roentgen-ray findings are characteristic and diagnostic for the disease. They may be summed up as follows:

1. Multiple, mostly intraperiosteal fractures, often showing areas of bone resorption at the seat of fracture.
2. Excessive callus formation.

23. Chaussier: Sur les fractures et les luxations observées chez le fœtus, etc., *Beitr. z. path. Anat. u. z. allg. Path.*, **19**, (after Feldmann, Ueber Wachstumsanomalien der Knochen).

24. Cortes: L'osteopsatirosis di Lobstein, *Zentralbl. f. Chir.*, 1910, **38**, No. 43.

25. Doering: Beitrag zur Lehre von der idiopathischen Osteopsathyrosis, *Deutsch. Ztschr. f. Chir.*, **77**.



3. Deficient shadow formation seen in all bones of the body, due to increased permeability to Roentgen rays. Often the bone shadows show but little more density than the surrounding soft parts.

4. The diaphyses of long bones may be slender, and only very rarely show any curvature or bending.

5. The cortex is of irregular thickness, on the whole very thin and parchment-like in appearance and may even appear to be absent in some places. There is little or no tendency toward thickening on the concave side of the shaft.



Fig. 1 (Case 1).—Osteogenesis imperfecta, C. R., aged 16 months.

6. The spongiosa contains wide meshes and an absence of structural markings. These changes are not limited to the diaphyses. All bones show this change, but not to the same degree, the most marked changes being found in the bones of the hands.

7. The medullary cavity is increased in size and shows irregularly mottled shadow.

8. The epiphyseal cartilages and their centers of ossification are larger than normal and the epiphyseal lines are straight.

The following cases of osteogenesis imperfecta came under our observation:

CASE 1.—C. R., aged 16 months, was admitted to the service of Dr. I. A. Abt, Michael Reese Hospital, May 10, 1914. Two months before, the mother had noticed that the child was losing markedly, getting weaker and the extremities becoming tender. The child could not stand even when held. She screamed when the limbs were touched and when she was being clothed. The abdomen was distended and the bowels constipated. During the previous month the spine had been turning. There was no history of past illnesses except that of gastro-enteritis. Her personal history revealed that she was one of twins, spontaneous birth. She sat erect at 9 months, but did not learn to talk or walk. She was breast fed until 3 months old; in the second month two bottles a day were substituted for the breast, and at 3 months she was put on the bottle entirely. The family history is negative, but both parents looked weak and emaciated.

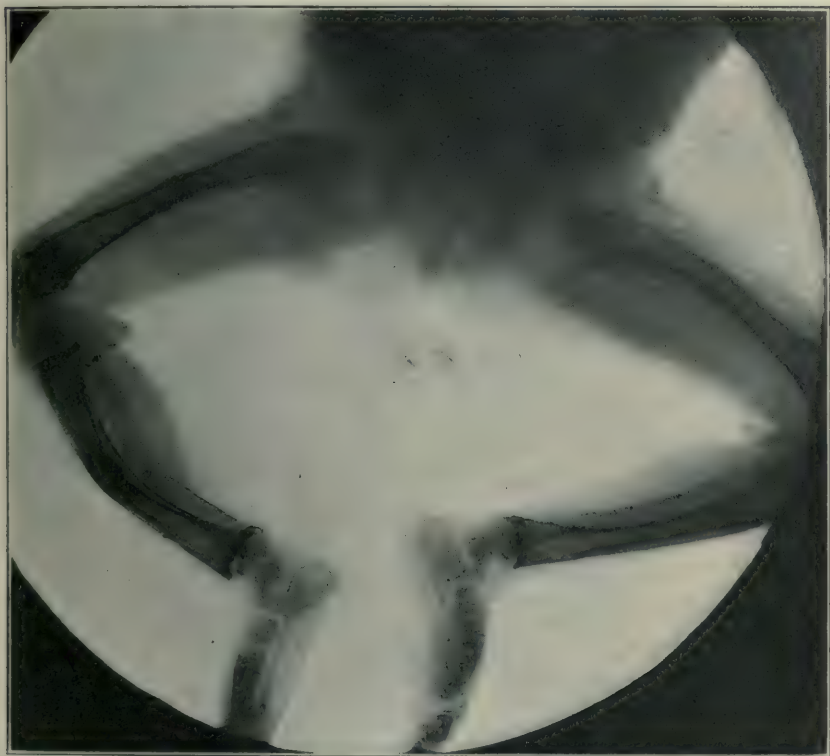


Fig. 2.—Same patient as shown in Figure 1, taken seven weeks later, showing marked improvement.

Examination showed her to be a fairly well nourished child who cried when touched. The lower extremities were drawn up on the abdomen. The head was square shaped and showed marked craniotabes. There was a marked rosary on the ribs and a flaring of lower costal margins; the lung findings were negative. The heart was negative. The abdomen was large and protuberant, somewhat distended. The spleen, liver and kidneys were negative. Lower extremities were flexed, the thighs on the abdomen, the legs on the thighs. The toes were flexed and the arches higher than normal. Any movement caused a shrieking cry as if in pain. There was considerable swelling, which

did not pit on pressure, but there were no redness and no fluctuation. There was discoloration over the middle of the left tibia and the upper part of the right thigh. There was epiphyseal enlargement of all the extremities. There was dorsal scoliosis. On May 12, 1914, the Roentgen-ray report (Fig. 1) showed fracture of the right fibula and femur and the left tibia and long fibula. The femur was very thin in the central third. The epiphyseal ends of the long bones and the tarsal bones showed no definite points of ossification. There was fracture of the radius and ulna, right and left. On July 21, 1914, roentgenograms were taken, which showed a definite amount of lime deposits in lamellae of bones. At the middle of the shafts of the left tibia there was a fracture; at this point there was deficient lime deposit. There was a distinct bowing forward at this point. There was a fracture with callus formation in the upper third of the shaft of the right radius and a similar condition in the ulna of the left arm. A roentgenogram taken Sept. 2, 1914, showed the same findings as before. On Oct. 6, 1914, the patient was getting along well, was gaining in weight and was looking well.

Summary of Roentgen-ray findings showed multiple intraperiosteal fractures of the shaft of the long bones with considerable callus formation. Bones showed little tendency to bend before fracture. There was also evidence of bone absorption at the seat of some of the fractures. There was absence of thickening on the concave side of the long bones; the epiphyseal line, how-



Fig. 3 (Case 2).—Osteogenesis imperfecta tarda, V. K., aged 3 years.

ever, was somewhat irregular, an evidence of rachitic changes. At the time of the discharge of the patient from the hospital, five months after she entered, the fractures showed a marked improvement, as evidenced by Figure 2, and the bones showed increased calcium deposit and the bone condition was in the stage of repair.

CASE 2.—V. K. was admitted to Cook County Hospital Nov. 28, 1914. The diagnosis was osteogenesis imperfecta tarda. The child was brought to the hospital by the father because the boy was unable to use his arms and legs. The father thought this began about a year before; previously to that he had been able to walk. The child had never been seriously sick at any time. The feeding history of the boy was not obtained.

The family history showed the father and mother living and well. There was another child 2 years of age brought to the hospital at the same time with a similar trouble, which had come on five months before. There were also one child at 4 years and one at 1 month living and well.

Examination showed a poorly nourished boy of 3 years who appeared below par, both mentally and physically. The head was large, appeared slightly hydrocephalic, and the hair was long and silky. The teeth were all present, but poorly formed. There was cervical adenopathy. There was flaring of the chest in the lower portion. A rosary was present, and there were nodules at the seat of the costal fractures. The lung findings and heart findings were





Fig. 4.—Same as shown in Figure 3, at the time of entrance.



Fig. 5.—Same as is shown in Figure 3 (Case 2), six months after treatment, showing a marked improvement.

negative. There was potbelly. The liver and spleen were negative. The extremities showed marked bend in the lower arm and of the femur. There was enlargement of the epiphyseal end of the femur at the knee. The upper portion of the fibula was freely movable and the shaft appeared to be cartilaginous. Both knee joints were very loose and the tibia could be moved laterally at the knee joint. The movements of the legs were painful. The musculature all over the body was flabby, but there seemed to be no complete loss in motion in any group. The knee jerks were increased.

Roentgenograms of the bones in Case 2 (Fig. 4) show the bones to be of less density than normal, and they appear thin and atrophied. The greatest density is shown subperiosteally in the shafts as a thin, almost paper-like layer, while the medullary cavity seems to be increased at the expense of the spongiosa. The epiphyseal lines are sharp, but perhaps less regular than normal. The concave side of the shafts of the long bones show an absence of thickening, which is usually markedly in evidence in rickets. The fractures are numerous and in most cases occur near the middle of the shafts and show large deposits of callus with little tendency to ossification, although there is a tendency to calcification. The bones as a whole show increased radiability



Fig. 6 (Case 3).—C. S., aged 2 years, osteogenesis imperfecta.

and appear unduly translucent. Some of them appear to have but little increased density over the soft parts. Six months later, while still under treatment, internally as well as by the orthopedic department, the child was again examined by means of the Roentgen ray and the general condition of the bones was greatly improved, as evidenced by Figure 5.

CASE 3.—C. S., was admitted to Cook County Hospital Nov. 25, 1915, and died Jan. 9, 1916. The diagnosis was osteogenesis imperfecta tarda with hydrocephalus and secondary rachitic changes, with a complication of bronchopneumonia. There was no history obtained except that the child had always been ill. The father said that the enlargement of the head had been present from birth. The complaint at the time of entrance was loss in weight and poor condition. The father was well and the mother was sick with pneumonia. There were five children, aged 9, 8, 7, and 3 years and one 8 months old, all well. There had been four miscarriages. No feeding history was obtained.

Examination showed an emaciated child of about 2 years, with several bone deformities. The hair was lusterless, uneven, long and fluffy. The head was hydrocephalic, square, with prominent parietal bones, and sweating. The eyes were bulging, pupils equal and reacted to light and accommodation. The mouth contained badly discolored teeth, just appearing through the gums, also an inflammation extending around the entire course of the gums and

tongue. The tongue presented many small spots of ulceration. The thorax showed a rosary on both sides anteriorly; the left thorax was larger than the right. The ribs were bulging on the left side posteriorly. There was kyphosis of the dorsal and lumbar regions, and lordosis of the upper dorsal region. There was potbelly, tympanitic, but no tenderness. All the bones of the extremities were affected. Both femurs showed fractures with a subsequent contraction of both legs and shortening of the hamstring tendons. There was a marked contraction of all the bones of the arms and legs, the clavicles have even seemed to have fractured (three fractures are visible in the roentgenograms). The patellar and Babinski reflexes were positive. Râles were heard throughout the chest; there were no areas of consolidation, no bronchial breathing. The heart tones were rapid, but there were no murmurs.



Fig. 7 (Case 3).—C. S., aged 2 years, osteogenesis imperfecta.

The Roentgen-ray findings on Dec. 2, 1915 (Fig. 7), show that the entire bony skeleton of this patient was involved in a high-grade osteo-atrophia; the long bones are very delicate in appearance, comparatively little distinction being noted between cortex and medulla. The former is, however, visible as a thin paper-like layer. The epiphyseal lines are (excepting the wrists) normal. Numerous intraperiosteal fractures are noted, the following bones being involved: left humerus three, left radius one, left ulna one, right humerus two, right ulna one, right radius one, right femur two, right fibula one, left femur one, a total of thirteen fractures. There is a peculiarity at the site of fractures noted which represents a ring of absorption of bone salts, but with definite callus formation surrounding these areas; the spine is seen deflected to the left to the middorsal region. The child died of bronchopneumonia Jan. 9, 1916.



CASE 4.—T. R., aged 3 years (Fig. 8), was admitted to the Cook County Hospital June 8, 1916, on the service of Dr. May Michael. A diagnosis was made of *osteogenesis imperfecta tarda*, with a bronchopneumonia. The patient is still in the hospital at the time of writing. He has had a cough ever since he was 6 months of age. He was at the Home for Crippled Children one month for advice. The child was born spontaneously at full term at Cook County Hospital, May 9, 1913. The first teeth appeared at 9 months. The child never walked. He was breast fed till nine months before entrance, then mixed diet was given. The family history showed the father and mother to be well; there were three other children, 1, 3 and 6 years of age, respectively, all well. Physical examination showed a poorly developed boy. The head was large, square and of a rachitic type. The eyes, nose and ears were normal. The lips were dry and cracked. The tongue and buccal membranes were sites of stomatitis. The pharynx was red. The chest was of a narrow lateral dimension, showing a rosary and marked flaring costal margins. The lung resonance was normal. There were a few coarse râles over the left lower lobe, but there was no bronchial breathing. The heart was normal. There was potbelly. The liver and spleen were not palpable. The extremities showed enlargement of the epiphyses on both lower and upper. On June 15, 1916, Roentgen-ray findings (Fig. 9) showed the case to be *osteogenesis imperfecta* and the lungs suggestive of tuberculosis. On June 23, 1916, roentgenograms



Fig. 8 (Case 4).—*Osteogenesis imperfecta*, T. R., aged 3 years.

showed the chest very narrow and the pulmonary area considerably increased in shadow; this shadow may or may not indicate tuberculosis. Examination of the extremities revealed that the long bones were very narrow as compared with their length; the shadow indicates very delicate bone structure; there is very little distinction between cortex and medulla; multiple fractures are seen to involve practically all of the long bones. A noticeable feature is an absorption of calcium at the seat of fractures. The bones are not curved as in rickets. The epiphyseal lines are more or less negative and differ greatly from the appearance usual in rachitis.

Of the four cases of *osteogenesis imperfecta* above described, two are of boys and two of girls. Their ages were 16 months, 3, 2 and 3 years, respectively.

#### DIFFERENTIAL DIAGNOSIS

The Roentgen ray is invaluable in the differential diagnosis of *osteogenesis imperfecta*, and also *chondrodystrophia foetalis*, cretinism, mongolism, rachitis, infantile scurvy, syphilis (early congenital and later manifestations) and tuberculosis of bones.

In *chondrodystrophia foetalis* the trunk is normal, while the



Fig. 9 (Case 4).—Osteogenesis imperfecta, T. R., aged 3 years.

extremities are shortened and deformed. In the skull the clivus is often seen in the lateral position and there is usually malposition of the nasal bones. If the root of the nose is retracted, they are almost horizontal, while if flattened, they are more vertical. The flattening of the bridge of the nose is due either to a primary tribasilar synostosis or a failure of development, which causes the base of the skull to be shorter. The ribs are thickened at the epiphyseal lines. The extremities are short and thick and the epiphyseal regions prominent.



Fig. 10.—Chondrodystrophia foetalis, N. F., aged 2 years.



Fig. 11.—Radiogram of chondrodystrophia foetalis. Same patient as shown in Figure 10.



The center of the body is displaced upward. The shafts of the bones often show great thickenings and shortening of the cortical substance, and overgrowth of the bony epiphysis is so marked that it often appears to overlap that of the epiphyseal line. The periosteum shows great thickening, the cortical substance is broader and thickened and the medullary canal is narrow, and sometimes the latter is replaced by hard cortical tissue. Thus it will be seen on examination of Figures 10 and 11 that the bone changes are marked in both the length and breadth. "The shortening of the long bones is due to the cartilaginous ossification of the epiphyses, and it is very evident that these abnormalities are brought about by the disturbance in the normal process of ossification in the primary cartilage" (Rotch).

In cretinism the body length is normal at birth, but shows later a greatly retarded development. The degree of retardation varies with



Fig. 12.—Cretin, aged 16 months. On superficial examination it shows some resemblance to osteogenesis imperfecta. However, the Roentgen-ray findings (Fig. 15) make the diagnosis complete.

the degree of thyroid atrophy (directly), and the amount and length of time of treatment. There is a universally retarded development of the bony skeleton due to delayed endochondral and periosteal growth, which is especially marked in the carpal and tarsal bones and in the phalanges. There is delayed development of the bony nuclei in the epiphyses and delayed union between epiphyses and diaphyses. The delays in bone deposits are usually very marked. A 2-year-old child may show bone development of a 6 or 12 month infant.

In mongolism Roentgen-ray examination has no great practical value, as in marked cases clinical diagnosis is easy and the bone changes have no definite pathognomonic characteristics. The general skeletal development shows no pathognomonic changes, the degree of retardation varying in different cases and different regions in the same case, while others may show no retardation. On the whole, the bones are well developed for their age, in fact occasionally the short bones

are developed too early. The skull is usually microbrachycephalic, the orbits low and converge inward, the sella turcica small, with overlapping clinoid processes, which often appear to involve the hypophysis. The hands are short and broad, due to small metacarpal and phalangeal bones. The thumb and little finger show the shortening to the greatest degree. The little fingers often show concavity on the side approximating the ring finger. This is chiefly due to shortening and curving of the middle phalanx. The thumb often shows a button like appearance with circular-like contraction at the proximal end of



Fig. 13.—Mongolian idiot, aged 1 year. See Roentgen-ray findings, Figure 16.

the metacarpal and distal end of the basal phalanx. There is a tendency to congenital anomalies, among which bone deformities are common; clubbed feet, syndactylism, superfluous toes, spina bifida, and micromelia of the upper extremities are often associated with other congenital deformities, as of the heart, genito-urinary tract and ears.

In rickets partial or complete fractures are usual, but they are not intraperiosteal and there seems to be very little tendency toward callus formation, although apparently enough to make the bone more or less solid. The calcium content of the callus is so very low that it is almost invisible in a roentgenogram. Deformities in rickets are much more



Fig. 14.—The Mongolian idiot and cretin, each about 2 years of age, showing the marked difference in physical development between these two conditions.



Fig. 15.—Radiogram of cretin illustrated in Figure 12, showing delayed epiphyseal ossification.



marked in the lower than in the upper extremities. Bowing and bending is characteristic. In the femur the greatest change may be in the neck, causing coxa vara. The pelvis may become flattened and the body shortened as a whole. Not infrequently palpable nodular thickenings involving part of the cortex are seen on the convex surface of the long bones. Increased radiability of bones is a property which rickets in its active stage shares with osteogenesis imperfecta. After the process in rickets subsides the bones, while still showing deformi-



Fig. 16.—Radiogram of Mongolian idiot shown in Figure 13. Normal epiphyseal ossification.



Fig. 17.—Mongolian idiot, showing curving of little fingers.

ties and curvatures, appear broader than normal and show even increased density of bone, which is especially marked at the ends of the diaphyses. The periosteum in rickets is definitely thickened, but the periosteal bone shadow is often pale, due to the low calcium content. The cortex has about the same appearance as in osteogenesis imperfecta. On the concave sides of long bones the cortex is thickened, which is almost pathognomonic of rickets; it may be thinned on the convex side. The medullary cavity is often increased in size in rickets and constantly so in osteogenesis imperfecta. The epiphyses,

which in osteogenesis imperfecta are normal, show most marked changes in rickets. The zone of proliferation is widened, with an irregular, toothed appearance on the epiphyseal side of the diaphysis, and there is also a broadening and flaring out of the epiphyseal end of the diaphysis, which is very characteristic for rickets. The epiphyses tend to be larger than normal, irregular in outline and may be of decreased density. The end of the shaft is streaked and presents an irregular line at the joint end and above it a curious transverse zone of different structure from the rest of the bone.



Fig. 18.—Acute rickets, first stage, showing thickening of cortex, pale spongiosa, cupping and fraying of the ends of the shafts, with poorly developed epiphyses and haziness of joints.

Lovett<sup>26</sup> divides bone lesions of rickets into three stages. As only the severe types of rickets call for differentiation from osteogenesis imperfecta and only from the late type of the latter, we will describe only the severe lesions, as noted by him. In the first stage the epiphyses cast little or no shadow, while the center of ossification is small or absent and at times appears multiple. The diaphysis becomes frayed out, instead of clear cut, the periosteum thickened, and the

26. Lovett: The Roentgenographic Appearances in Rickets, with a Comment on Differential Diagnosis, Jour. Am. Med. Assn., 1915, **65**, 2062.

whole joint appears hazy. Multiple fractures are common. This is the stage which is frequently classed as osteogenesis imperfecta (Fig. 18). In the second stage the shadow of the epiphysis becomes more marked, the area is ragged and irregular, the ends of the diaphysis begin to broaden, especially on the side on which the strain is greater, and here produces a ledge or lip next to the epiphyseal line. There is a thickening on the concave side of the shaft, which is a compensatory



Fig. 19.—Rickets, second stage, showing marked thickening of periosteum, flared and toothed appearance of ends of shafts, with increased bony formation in the nuclei.

change. The diaphysis begins to give a more definite shadow. The ends next to the diaphysis are streaked longitudinally and are the area of maximum disturbance. At the epiphyseal end of the shaft there is generally a transverse area of increased density, reaching about a quarter or half an inch from the epiphyseal line. This continues into the third stage. Further changes in the second stage consist in the chambering of the interior of the bone, where light areas in the shaft



indicate the absence of marked bone deposit, and heavier lines of ossification show the irregular development of trabeculae. The second stage is generally a period of systemic reaction to the disease, in which signs of returning ossification occur and when deformity begins (Fig. 19). In the third stage, the epiphysis begins to resume its normal contour and homogeneous shadow density. Irregularities persist in the marginal outline, and there is still a little mottling in the ossification. The lipping of the diaphyses has enlarged the bone ends, and



Fig. 20.—Rickets, third stage. Shafts show marked and irregular thickening, periosteum thickened on the concave side, disappearance of flaring and irregularities at the ends of the shafts and epiphyses distinctly ossified; marked curvature of the shafts.

there is in consequence a discrepancy in breadth between the diameters of the diaphysis near the epiphyseal line and the epiphysis (Fig. 20).

In infantile scurvy the most constant finding is an irregularly circumscribed shadow of varying diameter and intensity at the end of the diaphysis at the seat of new bone formation. This shadow, according to Rehn<sup>27</sup> enables one to make an early diagnosis of scurvy when all

27. Rehn: Röntgenaufnahmen von mit Lues congenita und Rachitis affizierten Knochen, Naturforscherversammlung, Düsseldorf, 1898.

other classical clinical signs of scurvy are absent, provided it is possible to exclude congenital syphilis. Less constant than this shadow are the subperiosteal shadows at the seat of hemorrhages. These shadows may be invisible even at the height of hemorrhage, becoming more and more distinct with age, probably due to the deposit of osteophytes. Fractures and infractions are demonstrable in scurvy. Other findings in scurvy, which, however, are not always demonstrable by Roentgen rays, are epiphyseal separation and displacements, hemor-



Fig. 21.—Infantile scurvy, showing bone atrophy, periosteal disturbance in shaft and so-called white line at the end of the shaft. This case also presents marked rachitic changes.

rhages within the joint capsule (considered to be very rare by Reyher,<sup>28</sup>) and occasionally, but rarely, intramuscular hemorrhages may be demonstrated. The clinical symptoms often enable one to make a diagnosis of scurvy and, according to Frangenheim,<sup>8</sup> especially in older children a diagnostic triad makes diagnosis comparatively easy;

28. Reyher: *Das Röntgenverfahren in der Kinderheilkunde*, Meusser, Berlin, 1912.

(1) hemorrhagic diathesis, which is manifested by tendency to hemorrhages and multiple hemorrhages anywhere in the body, with its sequela, marked anemia; (2) severe pain in extremities accompanying every movement; (3) swelling of the joints. From these signs multiple subperiosteal hemorrhages are pathognomonic.

In syphilis we must distinguish between the early congenital (Fig. 23) and the later bone changes (Figs. 24 and 25). As to the diagnosis of syphilitic lesions of the bones, Fraenkel<sup>29</sup> says: "If in an infant in its first weeks of life we find roentgenologically at the epiphyseal line either homogeneous transverse shadow or a streak interrupted by lighter transverse streaks, the epiphyseal line being well outlined

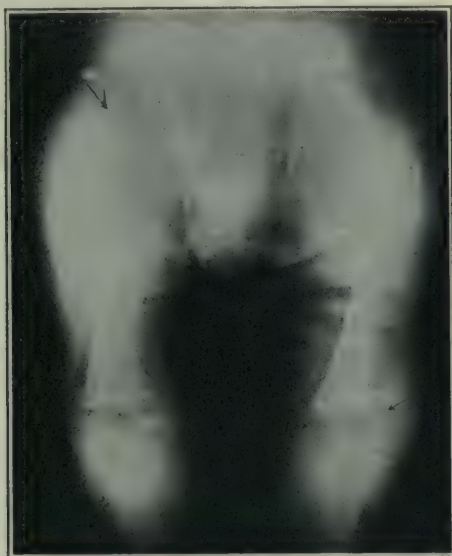


Fig. 22.—Infantile scurvy, showing epiphyseal separation or displacement due to hemorrhage into the joint.

toward the diaphysis, and toward epiphysis a serrated ribbon-like shadow, we have a positive proof of existence of osteochondritis syphilitica" (See our case, Figure 23). In early congenital syphilis the entire skeletal system is affected, the long bones suffering chiefly. In later manifestations, in which periostitis ossificans is a very important finding, we see newly formed subperiosteal bony masses with bony structure. They are most marked in the middle of the diaphysis of the femur and less marked in other long bones. Old cortex may often be differentiated from the newly formed subperiosteal bony masses. Cortical thickening is therefore an important point in dif-

29. Fraenkel: Die Röntgendiagnose der kongenitalen Knochensyphilis, Vortrag geh. auf dem VII. Kongress der deutschen Röntgen gesellschaft, Berlin, 1911.



ferential diagnosis from osteogenesis imperfecta, in which disease the cortex is always very thin. In the phalanges the epiphyseal line is somewhat lighter, the diaphysis is stronger and more compact and forms a dark, sharply circumscribed shadow. The phalanges are longer and thickened. There are definite changes in the exterior of the bone without any change in the interior, unless suppuration has taken place and other long bones of the body are similarly affected, which differentiates syphilitic dactylitis from tuberculous dactylitis. Fractures are comparatively rare in syphilitic infants (Lovett<sup>26</sup>).



Fig. 23.—Early congenital syphilis, showing marked changes throughout the entire bone structure.

A characteristic of tuberculous lesions is primarily and definitely a destructive local process, without evidence of a tendency to bone stimulation. Osteogenesis, on the other hand, appears to be a general atrophy of all the bones of the body. In tuberculosis light shadows, due to bone destruction and calcium absorption, are seen surrounded by otherwise normal dark bone shadows (Fig. 26), while in osteogenesis all the bones of the body show more uniformly increased permeability to Roentgen rays. The neighboring bone areas show

atrophy, the cortical substance is thinned and the spongy portion appears less dense, often giving a shadow of a density but little greater than the soft tissues. Callus formation in tuberculosis is very deficient. When tuberculosis affects joints, there is a high-grade atrophy and destruction of the bone. The bone ends show marked transparency. This increases the nearer it approaches the joint (Reyher<sup>28</sup>), thereby decreasing and often impairing the visibility of the joint contour. The entire region of the joint may become less visible. Comparison should be made with the well side, especially early in the disease, when diagnosis may be difficult. The tuberculous joint lesions



Fig. 24.—Late bone changes in congenital syphilis.

and fractures are very painful, thus differing from fractures of osteogenesis imperfecta, which seem to be attended with but little pain.

Osteomyelitis presents an outline showing periosteal reaction in the form of proliferation of new bone about the necrosed area, or again the sequestra may be plainly visible (Fig. 27). It is difficult to make positive diagnosis before the second week. By the second week we have evidence of a more or less marked periosteal proliferation and new periosteal bone formation about the necrosed area. Light and irregular shadows at the seat of the bone involvement are also now visible, due to bone destruction. Later in the disease distinct bone sequestra surrounded by a definitely lighter shadow are characteristic.

Before concluding our discussion we desire to point out the tendency on the part of Cases 1 and 4 of our series to develop secondary rachitic bone lesions. These rachitic changes led to a questionable diagnosis in two of our cases; however, a brief review of the changes characteristic of the two conditions will, I believe, clear up the situation. The combination of the two conditions in the late form of the



Fig. 25.—Congenital syphilis with later bone changes of periostitis ossificans.

disease is the rule rather than the exception, while the early congenital type has not infrequently been described as fetal rickets.

Most of the cases are sent into the wards as scorbutus, because of the pain at the seat of the fractures following motion of the extremities; the roentgenograms, however, readily show the cause of the pain.



Segawa<sup>15</sup> describes for the first time the combination of osteogenesis imperfecta as a congenital affection with Barlow's disease and rickets as acquired affections in an infant. The former was diagnosed clinically, the latter two only anatomically.

#### PROGNOSIS

The prognosis for infants affected with osteogenesis imperfecta is not favorable. A great majority of these children are stillborn or they die a short time after birth or within the first three years of their



Fig. 26.—Tuberculosis of the bones.

life. Since the disease is caused by a congenital anomaly, prognosis depends on the intensity of this anomaly, which may be judged from the time of its appearance and other clinical symptoms. Besides, these children are very susceptible to secondary infections and they usually die of some intercurrent disease. Prognosis is more favorable for cases occurring later in childhood as some seem to undergo spontaneous cure.

In the treatment of the disease calcium salts, phosphorus, and cod liver oil should be given a thorough trial. We have seen but little result from the use of desiccated thyroid parathyroid thymus, supra-

renal, anterior lobe of the pituitary gland and pineal gland, which have all been tried alone and in combination in our cases. Joachimsthal<sup>30</sup> reports a case in which by administration of phosphorus preparations he was able to produce within three months an almost normal



Fig. 27.—Osteomyelitis.

formation of bones and so complete healing of fracture that roentgenograms taken after that time showed no traces of the disease. Immobilization, with moderate extension of the fractures by casts, prophylaxis against further fracture, fresh air and sunshine, when

30. Joachimsthal: Heilungsvorgänge bei Osteogenesis imperfecta, München. med. Wehnschr., 1912, Part 1, **59**, 1246.

combined with cod liver oil, phosphorus and the calcium salts together with a diet containing fresh fruits and vegetables have resulted in most striking results in our cases of osteogenesis imperfecta tarda.<sup>31</sup>

	gm. or c.c.
R Phosphori .....	0 01
Olei morrhuae .....	60
M. Sig.: 4 c.c. twice daily or	gm. or c.c.
Calcii phosphoric. tribasic. puriss.....	6
Olei morrhuae .....	60
M. Sig.: 4 c.c. twice daily.	

5514 Indiana Avenue.

31. In addition to the references already given, the following will be found of interest:

- Dieterle: Die Athyreosis unter besonderer Berücksichtigung der dabei auftretenden Skelettveränderungen, sowie der differentialdiagnostisch vornehmlich in Betracht kommenden Störungen des Knochenwachstums, Virchows Arch. f. path. Anat., 1906, **184**, 915.
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# HEREDITARY HEMORRHAGIC TELANGIECTASIA

WITH REPORT OF THREE FAMILIES AND A REVIEW OF THOSE  
PREVIOUSLY RECORDED \*

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In 1865 Babbington<sup>1</sup> reported an interesting family in which recurring epistaxis had been observed for five generations. The transmission was through both male and female, but no mention was made of telangiectases in any of them. The first family in which these telangiectases were found was reported eleven years later by Legg,<sup>2</sup> who described numerous small nevi on the face, forehead and trunk of his patient. They developed about his 41st year. These cases were followed by others reported by Chiari,<sup>3</sup> Rendu,<sup>4</sup> Osler,<sup>5</sup> Josserrand,<sup>6</sup> Kelly,<sup>7</sup> Hawthorne,<sup>8</sup> Parkes-Weber,<sup>9</sup> Phillips,<sup>10</sup> Waggett<sup>11</sup> and Ballantyne.<sup>12</sup> Rendu, however, was the first of these observers to associate the presence of epistaxis with multiple telangiectases as clinical manifestations of a distinct morbid entity. Henceforth the erroneous diagnoses of hemophilia, acquired angiomas from cirrhosis of the liver and hemorrhagic diathesis were no longer applied to this condition. Further interest in its study was aroused by the papers of Sir William Osler, whose first published article on it appeared in 1901. Most of these cases were carefully abstracted in 1909 by Hanes<sup>13</sup> when he reviewed the literature on this subject and accurately described two

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\* Read in part at the meeting of the American Climatological and Clinical Association, Washington, D. C., May 9, 1916.

1. Babbington: *Lancet*, London, 1865, **2**, 362. Recently Lane (*Jour. of Heredity*, 1916, **7**, 132) has described hereditary nosebleed observed in a family for three generations.

2. Legg: *Lancet*, London, 1876, **2**, 856.

3. Chiari: *Erfahrungen auf dem Gebiete der Hals und Nasenkrankheiten*, Vienna, 1887, p. 60.

4. Rendu: *Gaz. d. hôp.*, 1896, **49**, 1322.

5. Osler: *Bull. Johns Hopkins Hosp.*, 1901, **12**, 333.

6. Josserrand: *Bull. de la Soc. méd. d. hôp. de Lyon*, 1902, **1**, 244.

7. Kelly: *Glasgow Med. Jour.*, 1906, **65**, 411.

8. Hawthorne: *Lancet*, London, 1906, **1**, 90.

9. Parkes-Weber: *Lancet*, London, 1907, **2**, 160.

10. Phillips: *Proc. Roy. Soc. Med.*, 1908, **1**, Laryngological Section, p. 45.

11. Waggett: *Proc. Roy. Soc. Med.*, 1908, **1**, Laryngological Section, p. 70.

12. Ballantyne: *Glasgow Med. Jour.*, 1908, **70**, 256.

13. Hanes: *Bull. Johns Hopkins Hosp.*, 1909, **20**, 63.

additional families, so that fifteen were then reported.<sup>14</sup> Since then eleven more families have been described, including the three here detailed, so that this condition has now been found to exist in twenty-eight families.<sup>15</sup> Other, isolated, instances, with no further cases in any of their families, have been reported by Chauffard,<sup>16</sup> Kelly,<sup>17</sup> Fox,<sup>18</sup> Galloway,<sup>19</sup> Lack<sup>20</sup> and Adamson,<sup>21</sup> but I have omitted all consideration of them on account of their lack of any hereditary tendency toward this condition.

It has been defined as a hereditary affection, manifesting itself in localized dilatations of capillaries and venules, forming distinct groups or telangiectases, which occur especially on the skin of the face, nasal and buccal mucous membranes and give rise to profuse hemorrhage, either spontaneously or as the result of trauma (Hanes).

In its causation, a hereditary tendency is the only factor which is constantly present, although others, such as syphilis, alcohol and traumatism, have been irregularly reported. A history of syphilis, however, is only recorded in three instances, yet its unrecognized presence may probably have existed in a somewhat greater frequency. Stokes<sup>22</sup> recently has written an interesting article showing that syphilis, chronic plumbism, hyperthyroidism and nephritis may cause cardiovascular degenerative conditions which result in the formation of generalized telangiectasia. Alcohol, likewise, is a most inconstant factor, which is only thrice stated to have been present, and traumatism, of etiologic importance, is just as rarely seen, for it appears but two times in the histories of the twenty-eight families exhibiting this condition, although

14. In this enumeration the family reported by Jossierand was unintentionally omitted and Osler's second case seemed to be an isolated instance, not of the family type. Later another member of the family, similarly affected, was discovered. Gottheil's family was also omitted from Hanes' list. It was published shortly before Hanes' article appeared. Hanes' list includes Babbington's family, in which epistaxis was observed for five generations, but no mention is made of telangiectases in any of its members. It probably should be added to this group, but, on account of the absence of positive evidence, I have omitted it.

15. Laffont: *Presse méd.*, 1909, **17**, 763. Langmead: *Proc. Roy. Soc. Med.*, 1910, **3**, Clinical Section, p. 109. Audry: *Rev. de méd.*, 1911, **30**, 22. Osler: *Riforma med.*, 1911, **27**, 57. Van Wagenen: *Med. Rec.*, New York, 1912, **81**, 109. Sequeira: *Proc. Roy. Soc. Med.*, 1912-1913, **6**, Dermatologic Section, p. 128. Gjessing: *Hospitalstidende*, 1915, **8**, 1151. Hutchison and Oliver: *Brit. Med. Jour.*, 1916, **9**, 67.

16. Chauffard: *Bull. et mém. Soc. méd. d. hôp. de Paris*, 1896, **13**, Series 3, p. 352.

17. Kelly: *Proc. Roy. Soc. Med.*, 1908, **1**, Laryngological Section, p. 45.

18. Fox: *Brit. Jour. Dermat.*, 1908, **20**, 145.

19. Galloway: *Proc. Roy. Soc. Med.*, 1910-1911, **4**, Clinical Section, p. 42.

20. Lack: *Jour. Laryngol.*, 1909, **24**, 185.

21. Adamson: *Brit. Jour. Dermat.*, 1909, **21**, 219.

22. Stokes: *Am. Jour. Med. Sc.*, 1915, **149**, 669.

it must be stated, as Hanes has previously shown, that the telangiectases are generally located at points most subject to frequent slight traumatisms. The bleeding in these cases is often so severe that a condition of marked anemia is induced, which may be an important factor in the development of the telangiectases. Certain it is that the bleeding generally precedes the formation of these localized dilatations of the capillaries and venules, and may do so with many years intervening between them. In some of the cases, also, a respite from the bleeding appears to be coincident with the disappearance of the telangiectases. Cases have been reported in England, France, Germany, Denmark and the United States. The Anglo-Germanic race has furnished most of the examples, followed by the Latin and Scandinavian.

Kelly thinks that October to May, inclusive, are the months in which the bleeding is most apt to occur, but other observers have not corroborated this fact. Hutchison and Oliver thought their patient bled more during the summer months. It has been noted in ninety-two males and seventy-three females, so its distribution among the sexes is probably about equal. Both sexes are able to transmit it. In the published cases thirty-two women have transmitted the tendency as against thirty-five men. The pathology of this condition has been investigated on three occasions. The first case was when Dr. Mabel Austin examined at Dr. Osler's suggestion a section of the nasal mucous membrane of a patient, in the first family which Dr. Osler reported. This patient was the first to come to necropsy, which revealed "cancer of the stomach, mesentery and omentum, liver, retroperitoneal glands, lungs and brain." In the stomach there were also found "a dozen round foci, each from 3 mm. to 4 mm. in size, which at first looked like ecchymoses, but were dilated venules and capillaries." The sections of the septum of the nose showed large dilated veins just beneath the epithelium. Our information from the second case comes from a biopsy which Dr. Hanes performed on one of his patients. The tissue was removed from a telangiectatic spot and showed "the obliteration of the papillae of the corium, together with the absence of the usual undulations of the stratum germinativum. This is doubtless due to the relatively enormous dilatation of the blood vessels of the corium, which are seen as wide spaces, lined by a single layer of endothelium lying immediately subjacent to the greatly attenuated epidermis. These dilated vessels can be traced well down into the subcutaneous fatty tissue. A study of sections stained by various special methods failed to reveal any muscular or elastic tissue in the walls of the dilated superficial vessels, although the less dilated vessels in the subcutaneous fatty tissue show the normal arrangement of the tunicae." Consequently, Hanes refers to the insufficient protection of the dilated vessels and



states that it is not surprising that trivial traumatisms produce marked hemorrhage.

In one member of the family reported by Gjessing a microscopic examination was made from an angioma on the left cheek. In the corium blood-filled cavities were found, surrounded by a single layer of endothelium and a thin stratum of connective tissue, devoid of elastic or smooth muscle fibers. In some places the cavities extend upward to the epidermis, where the papillae were flattened. In one spot the cavity extended down into the subcutaneous tissue, where, in the surrounding area, numerous well-developed hair follicles and sebaceous glands were seen. In this situation the elastic fibers were as numerous and well developed as in normal tissue. The changes then consist in a dilatation of the capillaries of the small veins, with the formation of telangiectases of the three types which I shall later describe. These dilated vessels, being only lined by endothelium without the additional presence of elastic or muscle fibers, become consequently very liable to rupture, induced either spontaneously or by trauma.

The chief symptom is hemorrhage, which may come from the telangiectases in the nose as an epistaxis or from those situated elsewhere. These locations are the conjunctivae of the upper and lower lids, the ears, cheeks, nose, lips, mucous membranes of the mouth in the region of the hard or soft palate, the uvula, buccal mucous membranes or gums, tongue, neck, trunk, back, arms, finger tips, under the nails, or on the feet. The bleeding varies greatly in frequency. It may occur three to four times daily, once or twice a week or even with a greater interval of freedom. Kelly has described a patient in whom it was most apt to take place between the months of October and March, and would be both profuse and frequent. The amount of blood lost also varies greatly. In Kelly's patient, just referred to, it was so marked that death from syncope finally resulted. Legg and Chiari have reported cases in which dropsy ensuing from loss of blood caused a fatal termination, while Phillips has recorded a case in which death came from hemorrhage of the gums. Chiari also mentions a child, in one of his families, who died from a severe nosebleed, and Gottheil speaks of a male dying from the same cause.

The bleeding may be a slight trickle from the nose or the spots, or come with more force. One of Hanes' patients found it not uncommon for him to injure one of the lingual telangiectases while eating, so that the blood would actually spurt from the injured spot and render further progress with the meal impossible. Gottheil has described two varieties of bleeding in one of his cases. From the nose it occurred as a slow trickling, lasting perhaps ten or fifteen minutes, while from the visible lesions of the tongue and lips it came as a sudden projectile spurt,

sometimes reaching out a foot or two, if on the lips, or if his mouth was open, and stopping spontaneously in two or three minutes. Langmaid speaks of his patient as suffering for about twenty years from frequent epistaxis and adds that occasionally his face or tongue has burst out bleeding. Legg also speaks of the bleeding from the spots as being frequently spontaneous in origin in his case, but generally in all the instances it is the result of traumatism, while the hemorrhage from the nose is spontaneous in its onset.

Less severe bleeding has produced, by the resulting anemia, vertigo, headaches, weakness, dyspnea on exertion, palpitation and swelling of the ankles. In one instance fainting was induced after a prolonged epistaxis, while another patient, depleted from the hemorrhages, finally came down with an endocarditis. The prostration from this cause prevented another from working for about three years. The onset of the epistaxis is generally in early childhood, but the attacks become more severe and prolonged as the patients advance in years, the period between the 35th to 38th years being the time when the increase is especially noted. The telangiectases seen in this condition are of three varieties: the pinpoint, which is most apt to be seen on the skin of the hands and face, and which may be readily overlooked; the spider form, which is the most common and which Parkes-Weber prefers to call spider angiomas rather than spider nevi; and the nodular type, which may originate in the center of a spider angioma and finally form a solid vascular tumor, split pea in size. They are most frequently seen on the nasal and buccal mucous membranes and on the mucocutaneous junction of the lips, but may be found in the other locations mentioned above. They begin as capillary dilatations and are bright red in color. Later the venules give the cutaneous telangiectases a violaceous or purple color by participating in their formation. The spots on the mucous membranes, however, always remain a bright red. They may be seen early in life, for Hanes has observed them in a boy of 8 years, but generally, if present at such an age, they are not especially numerous, for they do not attain their full number until after the age of 35. Even then they appear and disappear with marked frequency and seem to bear some relation to the bleeding, being less marked if a considerable respite from hemorrhages is observed. Parkes-Weber speaks of a vicious circle being established by the repeated attacks of bleeding giving rise to a grave condition of anemia, which in its turn increases the tendency to hemorrhage.

In Parkes-Weber's case a small patch of chorioretinitis was found in an examination of the fundus of the right eye by the ophthalmoscope, while in Sequeira's case the retinal vessels were thickened, but there were no hemorrhages.

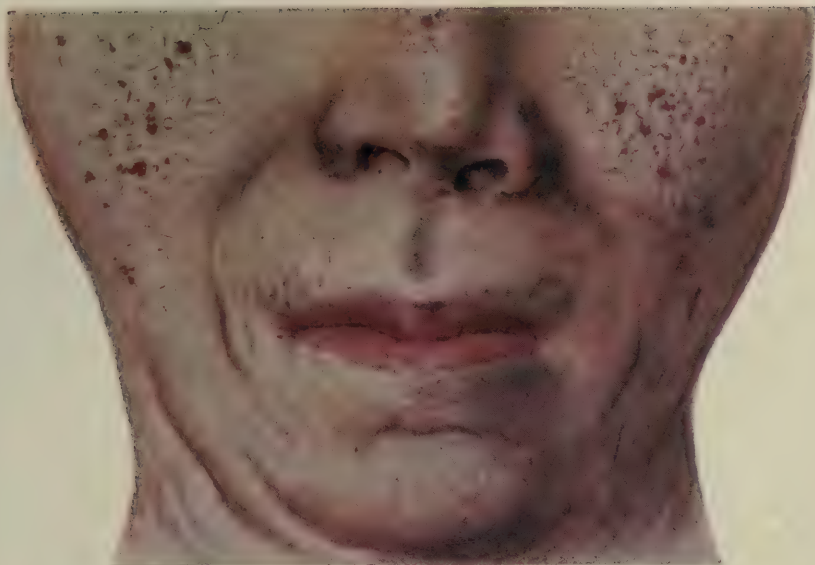


Fig. 1.—Telangiectases on the face. The family tree of this patient is given in Figure 3 (Family 28, Steiner 1, III, 22).

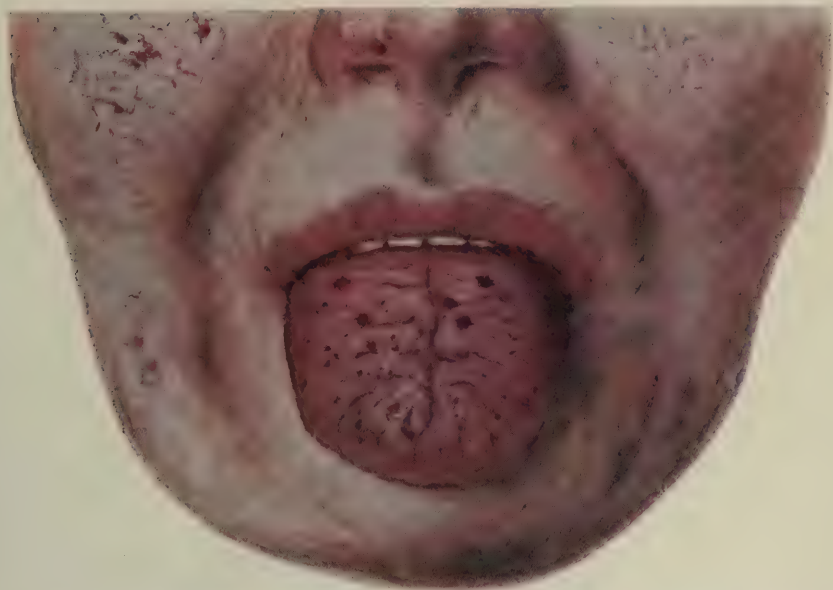


Fig. 2.—Telangiectases on the tongue. The family tree of this patient is given in Figure 3 (Family 26, Steiner 1, IV, 12).





Before Rendu's time the disease was generally considered hemophilia. Chiari, however, caught a glimmer of the real truth when he first diagnosed the condition as one of telangiectases of the mucous membranes, which were hereditary in a family. Unfortunately, after a further study of his cases, he regarded the affection as due to hemophilia. The epistaxis, or the telangiectases, may exist alone in certain members of the families implicated, but the hereditary tendency in them and the finding of the cardinal symptoms combined in other members of the family will make the diagnosis simple. The fact that the females are not affected by hemophilia may be, at times, of diagnostic value.

The outlook is generally not very promising, for the hemorrhages are apt to increase in severity as middle life is reached, when the telangiectases are frequently first seen. In one of the cases, however, there was a cessation of the bleeding as this period was approached, while another bled only after this time from her nose and the telangiectases elsewhere, at each menstrual period. In four out of the 191 cases death has been directly attributed to the hemorrhages, in others it has involved much invalidism, while a third and smaller group has not been much incapacitated by them.

Rendu used with excellent result, in his case, an application on the nasal mucous membrane of a powder of antipyrin, tannin and powdered sugar, and Coe has reported on the beneficial employment of calcium lactate and iron in his case, which he published as one of hemophilia. It is hard, however, to see the rationale of this treatment, for the disease is due to a developmental defect in the blood vessels and measures to increase the coagulability of the blood ought not to yield good results. The use of iron and the application of the thermocautery to many of the spots might more reasonably explain the patients' improvement. A bead of chromic acid, fused on a probe, was used with excellent success by Hanes, who recommends the method highly, as the action of the caustic may be checked at any time by the application of an alkali. The repeated use of this method may be necessary to check the bleeding. After the cause is thus removed, the use of iron and arsenic for the treatment of anemia is indicated. Although it has been unsuccessful in some cases, the cauterization of the troublesome bleeding areas appears to be the most satisfactory form of treatment. The use of radium for the nodular telangiectases might also be of avail.

Since my interest was aroused in this condition from a case on the services of Sir William Osler, at the Johns Hopkins Hospital, in 1899, I have seen instances of it in three families and can give the following partial rehearsal of their histories.

FAMILY 26 (Figs. 1, 2 and 3, Steiner 1, 1916).—Recurring hemorrhages occurred in five generations and twenty-one members of one family; telangiectases were observed in five of them.

F. G., aged 42 years (Hospital No. 66559), was admitted to the Hartford Hospital Feb. 23, 1913, complaining of weakness, shortness of breath and frequent nosebleed (Fig. 3, III, 4).

Family History: The patient's grandfather (Fig. 3, I, 1) was a bleeder, as well as his father (II, 1) and one aunt (II, 6) all of whom are now dead. Of the aunt's family of ten children, two sons (III, 12 and 14) and two daughters (III, 22 and 24) are bleeders. One of the daughters (III, 22, Figs. 1 and 3) has a girl (IV, 18) who is also a bleeder, and one son (III, 12) has two daughters (IV, 10 and 12) and a son (IV, 17) who bleed likewise. One of these daughters, both of whom are married, has a daughter (V, 6) who bleeds from the nose. The histories of III, 22 and IV, 12 (Figs. 1, 2 and 3) will be given later. Another aunt (II, 8) has two sons (III, 29 and 30) who are subject to epistaxis, and an uncle (II, 3) has likewise two sons (III, 6 and 7) who suffer from the same difficulty. The patient (III, 4) has three sons and a daughter (IV, 1, 2, 3 and 4) who are similarly affected.

Present Illness: The patient was in excellent health until he was 14 years old, when he began to suffer from frequent attacks of epistaxis, which were exceedingly difficult to control. They recurred weekly or at more frequent intervals. When he was 16 years old, he had a slight hemorrhage from a small red spot on his face, which was readily checked. Three years later he entered the Prussian army and suffered frequently from the symptoms mentioned above, although the amount of blood lost was never very great. There was never any additional hemorrhage noted from his mouth or rectum, and his urine never contained blood. He remained in a stationary condition during the four years he served in the army and in this period was observed by army physicians and others who told him the bleeding could not be checked. At the expiration of his army service he emigrated to the United States, where he was frequently in very straitened circumstances. Occasionally he found employment in the Hartford coal yards. After two years he returned to Germany again, where he labored successfully for thirteen years as a grocer and lumberman. Then meeting reverses, he came to this country again, four years ago, and has since worked as a pedler. During all this time he has had frequent attacks of epistaxis and has gradually grown weaker. For the past year dyspnea on exertion as well as palpitation have been troublesome symptoms. The hemorrhages generally occur in the mornings and of late have been observed daily. His appetite has been good. There have been no digestive disturbances. His bowels have been constipated, but have recently been moved daily by medication. Of late years he has suffered from hemorrhoids, which occasionally bleed somewhat. He had measles when he was a child. He denies venereal disease. He has been a moderate smoker and drinker.

Physical Examination: The patient was a well-nourished, well-developed Jew. The lungs were negative on examination, but the heart, which was not enlarged, revealed a soft systolic murmur at the apex, which was not transmitted outward, but was heard with increasing intensity on passing upward, being loudest at the base, in the pulmonic area, where the second sound was slightly accentuated. Over both cheeks, on either side of the nose, numerous angiomas are seen, varying in size from pinpoint to one which measures 3 by 4 mm. in size, located 20 mm. below a midline drawn perpendicularly from the left lower eyelid. Two pinhead angiomas are also seen on the nose, and on the mucous membrane of the lower lip ten more are noted from pinpoint to pinhead in size. On the tip of the tongue, two are also observed, the larger measuring 2 by 2 mm., while the smaller resembles a pinhead. Back of the wisdom tooth, on the right lower jaw, there is also one of a similar size, as well as on the buccal mucous membranes and on the posterior aspect of the tongue. Two small ones are likewise seen on the anterior aspect of the helix of the



right ear, while six more are visible on the posterior surface. On the septum of the nose, as well as on the left inferior turbinate, similar angiomas are observed and some of them appear to have bled very recently. The mucous membranes of the nose as well as the conjunctivae are very pale in color.

Blood examination showed red blood corpuscles 4,696,000; leukocytes 4,800; hemoglobin (Dare) 60 per cent.; coagulation time three and a half minutes (Biffi Brook's instrument). A differential count of 300 leukocytes gave polymorphonuclears, 61 per cent.; lymphocytes, 26 per cent.; large mononuclears and transitionals, 12 per cent., and eosinophils, 1 per cent. There were no myeloblasts, no normoblasts or megaloblasts, no poikilocytosis, and but slight anisocytosis. The urine examination was negative. On March 5 Dr. E. Terry Smith examined the patient's nose and observed the presence of an old atrophic rhinitis. He also noted some telangiectases on the septum and left inferior turbinate, which he cauterized on two separate occasions. His other treatment consisted in the use of rest, iron and arsenic. Three days before discharge the patient's hemoglobin was 70 per cent., but the blood examination otherwise showed no change. On April 19 he was seen with me by Sir William Osler, who confirmed the diagnosis. He was discharged from the hospital March 23.

The patient was then lost track of by me and could not be traced, as he had moved from the residence he gave, until April 1, 1916, when he walked into my office. After leaving the hospital he had worked steadily as a pedler until recently, when the hemorrhages from the telangiectases on his face, tongue and nose prostrated him so severely that he found himself in a very much weakened condition and unable to work. A few days later I had a Wassermann and luetin test performed on him, with negative results in both instances. The internal administration of iron chlorid has yielded him some improvement.

Mrs. A. T., aged 33 years (Figs. 1 and 3, III, 24), first seen by me at my office on March 20, 1909, complained of bleeding from the face. The family history is given in the recital of the history of the previous case. The past history shows typhoid fever when the patient was 24 years old, of nine weeks' duration, with complete recovery. Two years previously she injured her thigh severely in an explosion. Her menses have been regular, occurring every twenty-eight days, with a duration varying from three to seven days and with slight pain. She has been married for ten years and has had three children, the age of the eldest being 8 and of the youngest 3. The labors have been instrumental and difficult.

Present Illness: She has bled from the nose ever since she can remember. The epistaxis begins and stops spontaneously. The intervals between the bleedings have varied from three to four weeks up to two or three months. One year ago ergot internally seemed to check them. At this time she first began to bleed from the tongue, pharynx and hard palate. These hemorrhages are also well controlled by ergot and the local application of tincture of ferric chlorid. Ten days ago the bleeding began to be more profuse and would come on especially during eating, when mastication would frequently remove a clot from the tongue.

Physical Examination: The patient was a well-nourished, well-built woman. The lips and mucous membranes were quite pale in color. The tongue was clean. On the dorsum of the tongue, near its middle aspect and slightly to the right, was a raised nodular angioma, split pea in size, with a flattened summit, from the center of which a pinpoint opening of a blood vessel was seen. The mucous membranes of the hard palate showed a number of telangiectases, pinhead in size. They were also seen about the vicinity of the cheek bones, one on the left side of the nose to the right of the bridge and 1.5 cm. from the tip being of the nodular type and split pea in size. The others varied from pinpoint to pinhead in size. All were bright red in color and did not blanch on pressure. On the right hand, near the styloid process, was seen a spot pinhead in size. The heart revealed a soft systolic murmur at the apex,

which is not transmitted outward and was heard with increasing intensity on passing upward, being loudest at the pulmonic area. There was a soft systolic murmur in the vessels of the neck.

Blood examination showed red blood corpuscles, 4,304,000; leukocytes, 7,750; hemoglobin (Dare), 55 per cent.; blood platelets (Pratt's method), 204,788; coagulation time (Biffi-Brook's method), two minutes. Differential count of 500 leukocytes gave polymorphonuclears, 61.8 per cent.; lymphocytes, 26 per cent.; large mononuclears and transitionals, 9 per cent.; eosinophils, 2 per cent., and mast cells, 0.4 per cent. There were no myeloblasts, no normoblasts or megaloblasts, no poikilocytosis, and but slight anisocytosis.

Subsequently the bleeding increased in severity and frequency so that the patient has occasionally been much prostrated by the secondary anemia thus induced. The use of rest, iron and arsenic has been of little avail.

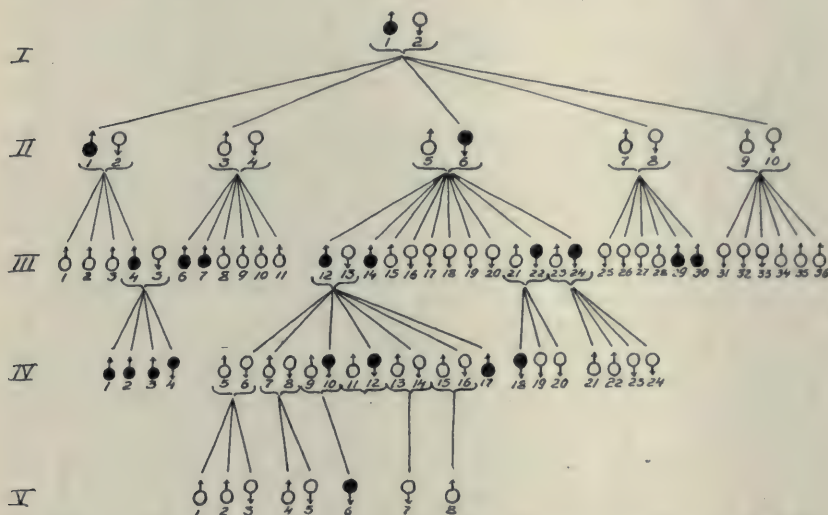


Fig. 3.—Family 26, Steiner 1. The heavily shaded characters in this and all succeeding figures indicate the members of the family affected, while the Roman numerals at the margin indicate the successive generations.

Mrs. D. E., aged 25 years (Figs. 2 and 3, IV, 12), was first seen by me at my office on April 26, 1909, complaining of bleeding from the nose. The family history is given in the recital of the history of the first case. The past history shows influenza every winter ever since she can remember. She had brain fever when very young and measles when she was a child. Her menses began at 14 and were regular every twenty-eight days until five or six years ago, since which time they have come a few days more frequently. She has much pain during the first two days.

**Present Illness:** Nine years ago she first began to bleed from the nose. The hemorrhages have come on since then five or six times a day, sometimes once a week or more rarely once a month, occasionally there is a two or three month interval. A little worry or excitement will generally bring on an attack, which frequently lasts five or ten minutes. She has never used anything to check the hemorrhages except cold water. Last summer she first began to bleed from the tongue and has bled thus about five times in all.

**Physical Examination:** The patient was a well-nourished, well-built woman. The lips and mucous membranes were of good color. Over the cheeks, chin, mucous membrane of the lower lip and the tongue were telangiectases varying



in size from a pinhead to split pea in size of which there were two on the tongue which were slightly raised. Some were of the spider type. All were cherry red in color. The heart was negative on examination. A Wassermann reaction on the blood and spinal fluid was later performed with negative results. Blood examination showed red blood corpuscles 4,000,000, white blood corpuscles 10,000, hemoglobin 75 per cent. The urine examination was negative. Subsequently the bleeding, especially from the nose, has increased in severity. The cauterization of the bleeding areas has been of little avail.

FAMILY 27 (Fig. 4) (Steiner 2).—Recurring epistaxis occurred in three members of the family for two generations; telangiectases with epistaxis were present in the only member of the family seen.

E. N., aged 41 years (Hospital No. 66927), was admitted to the Hartford Hospital on my medical service, March 17, 1913, complaining of being run down (Fig. 4, II, 8).

Family History: Her father had died of old age, her mother of pneumonia. Of her four sisters and one brother, one, a sister (II, 5), aged 47, had been subject to attacks of epistaxis all her life, but especially during childhood. She is married but has no children. She resides in Stockholm, Sweden. Another sister (II, 7) has a son (III, 7), aged 17, who has had frequent attacks of epistaxis since early childhood. There are no others in the family subject to epistaxis.

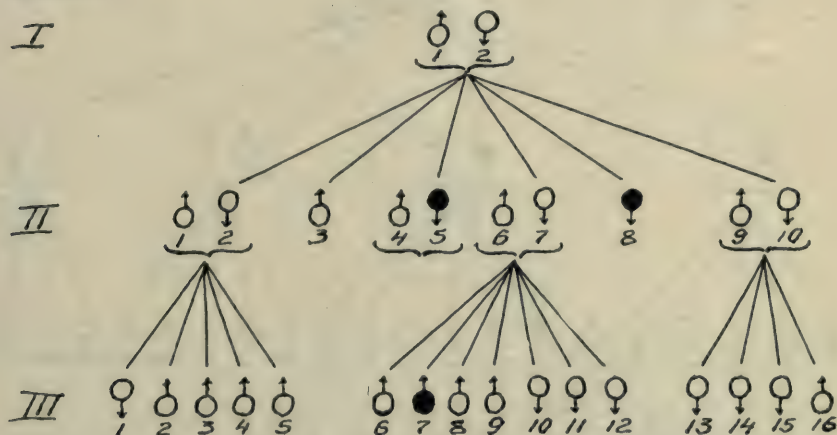


Fig. 4.—Family 27, Steiner 2.

Past History: The patient had measles at 8 years of age. Her menses began at 14, were regular every twenty-eight days, with a duration of six or seven days. The flow was always very profuse.

Present History: When she was about 25 years old, she began to have attacks of moderate epistaxis the day preceding the onset of each menstrual period. Her gums, however, have always bled easily when she cleans her teeth. Twelve years ago she emigrated to this country from Sweden, and two or three years later she began to have red spots on her cheeks. Two years later she noticed them on her hands and wrists. Occasionally she has had slight attacks of dizziness during the past few months. For the past week she has had one to three attacks of severe epistaxis daily, followed by sensations of weakness. The hemorrhages last from ten to fifteen minutes and cease spontaneously.

Physical Examination: The patient was a rather poorly nourished, slenderly built woman. The lips and mucous membranes were quite pale. Over the ears, cheeks, mucous membranes of the lips, tongue and cheeks, wrists,



hands and feet were telangiectases, mostly of the nodular type, pinhead in size, but some were of the spider variety. Some were also seen under the nails of the middle and index fingers and thumb of the left hand. The lungs were negative. The heart was not enlarged, but revealed a presystolic thrill and murmur at the apex, sharply localized. A systolic murmur was also here audible, which could be heard outward to the anterior axillary line and upward to the base, but in both directions with decreasing intensity. The pulmonic second was accentuated.

Blood examination showed red blood corpuscles 2,560,000; white blood corpuscles 5,200; hemoglobin (Dare) 55 per cent.; coagulation time four and a half minutes. Differential count showed polymorphonuclears, 69 per cent.; lymphocytes, 15 per cent.; large mononuclears and transitionals, 24 per cent., and eosinophils, 2 per cent. There were no myeloblasts and but one normoblast seen. There was marked anisocytosis and moderate poikilocytosis.

The patient refused to have the bleeding points in her nose cauterized. Her treatment consisted in the use of rest and iron. After a week's sojourn in the hospital, she left on March 24 to set sail for Sweden from New York on the following day.

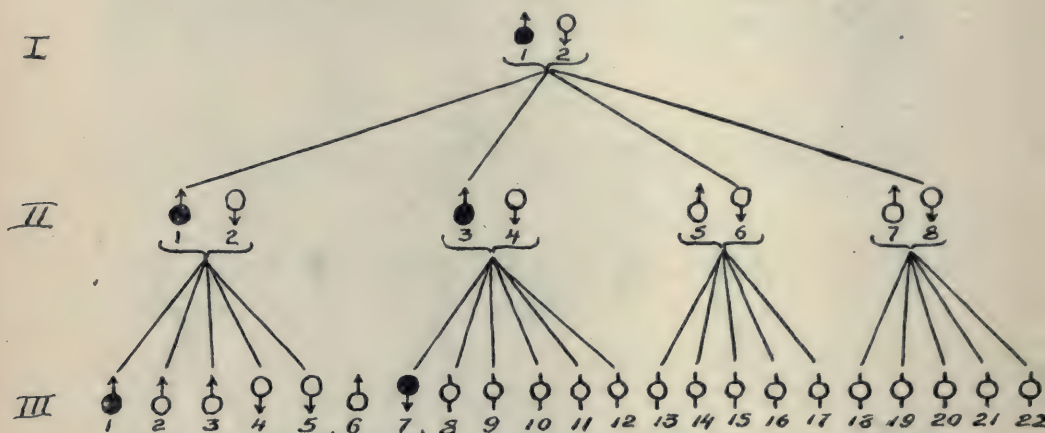


Fig. 5.—Family 28, Steiner 3. The character with the arrow stem both at the top and bottom of the circle in this family tree and in succeeding figures indicates that the sex is not stated.

FAMILY 28 (Fig. 5) (Steiner 3).—There was a history of epistaxis and telangiectases in the family for three generations.

C. L., aged 62 (Hospital No. 86348), was admitted to the Hartford Hospital on my medical service March 15, 1916, complaining of a cough (Fig. 5, II, 3).

Family History: His father (I, 1) who died at 92, had telangiectases and nasal hemorrhages, but further history about him was not obtainable. A brother (II, 1) had a similar condition, as well as his brother's son (III, 1). Of the patient's six children, a daughter (III, 7) suffers from occasional attacks of epistaxis.

Past History: The patient had always enjoyed good health. A few weeks before he entered the hospital, he had a slight operation on his tongue for what he says was a cancer. He had bled from the nose occasionally since boyhood, but recently the attacks had lessened in severity and frequency.

Present History: Four weeks ago he contracted a bronchitis and has coughed considerably ever since. With the cough he expectorates a little clear tenacious sputum. There are no other symptoms.

**Physical Examination:** The patient was a well-developed, well-nourished Italian. There was evidence of a slight operation on his tongue on the left side. The edges of the healed wound were not ulcerated but felt indurated. The teeth were in very bad condition and the breath was very foul. The lungs revealed medium, moist râles at both bases, posteriorly and in the left axilla. Over his forehead, cheeks and nose pinpoint, nodular and spider angiomas were noted. The largest one of these was located on the left cheek, 4 cm. below a line drawn from the middle of the lower eyelid and 1.5 cm. from the left ala nasi. Under the use of expectorants his cough lessened in frequency, became looser in character and finally left him. A Wassermann reaction was performed on his blood, withdrawn from a vein at his left elbow, with negative results. His teeth were cleaned by Dr. W. N. Butler, one of our dental surgeons, and the patient was discharged from the hospital in good condition on April 12, 1916.

**FAMILY 1 (Fig. 6) (Legg, 1876).**—There was a history of epistaxis in three generations; developmental telangiectases were noted on the patient's face and trunk, but there was no history of hemophilia.

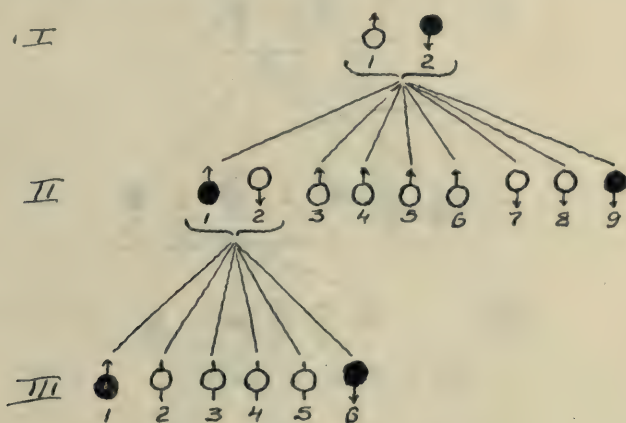


Fig. 6.—Family 1 (Legg).

The patient, II, 1 (Fig. 6), aged 65, gave a history of attacks of epistaxis since boyhood. He has also shown a tendency to bleed profusely from traumatic causes. His mother (I, 2) died from loss of blood and dropsy. One sister (II, 9) is subject to epistaxis. Of his six children a son, aged 27 (III, 1), and a daughter, aged 22 (III, 6), suffer from epistaxis. The patient has numerous small nevi over his face, forehead and various parts of his trunk which first appeared about his 41st year. Violent fits of anger as well as excessive drinking bring on attacks of epistaxis. When seen he had bled every day for six weeks.

**FAMILY 2 (Fig. 7) (Chiari, 1887).**—There was a history of epistaxis in four generations, with multiple telangiectases on the skin and mucous membranes, a condition diagnosed as hemophilia.

Two sisters (III, 5 and 7) stated that their grandmother (I, 3) and her two brothers (I, 1 and 4) suffered from childhood with frequent and severe attacks of epistaxis, yet lived to ripe old ages. Their mother (II, 2) also had the same difficulty and died at the age of 47 from dropsy, which apparently developed after a long period of nosebleed. Two brothers and one sister (III, 1, 2 and 3) were similarly affected with these severe attacks of epistaxis, but the sister had apparently recovered from these attacks, except at each menstrual period, when the epistaxis was insignificant. The two sisters mentioned above (III, 5 and 7) have bled since childhood from the nose, and one

of them once bled for a long time from the gums. Both of them have telangiectases on their faces and the upper part of their bodies. These are also seen on the mucous membranes of their noses, tongues and lips. III, 5 had four children, the youngest of whom, aged 4, bleeds from the nose. III, 7 had a son, aged 6, who has had epistaxis for four years.

FAMILY 3 (Fig. 8) (Chiari, 1887).—There was history of severe epistaxis in three generations, with multiple telangiectases on the skin and mucous membranes.

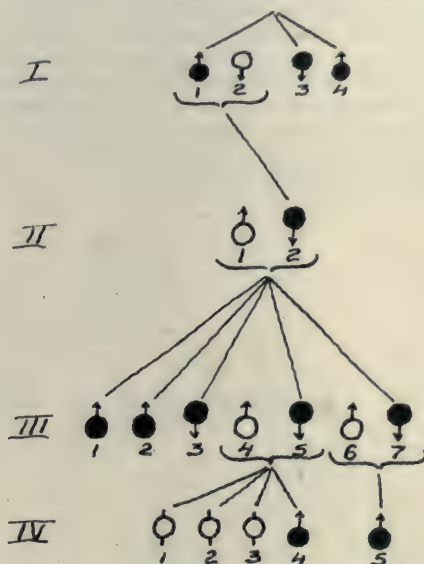


Fig. 7.—Family 2 (Chiari 1).

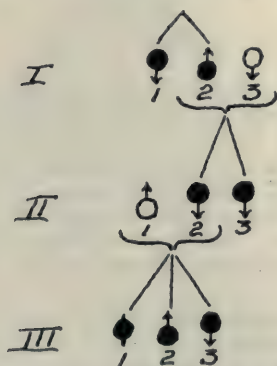


Fig. 8.—Family 3 (Chiari 2). The patient III, 1 died from severe epistaxis.

II, 3 had suffered from childhood with severe attacks of epistaxis. She was about 30 years old when first seen by Chiari and exhibited telangiectases on the skin and mucous membranes of the nose, tongue and lips. She stated that her father (I, 2) and her paternal aunt (I, 1) had had the same trouble. Her sister (II, 2) was likewise affected, as well as her sister's three children (III, 1, 2 and 3). One of them had died some years previously from a severe nosebleed.



FAMILY 4 (Fig. 9) (Rendu, 1896).—There was history of severe epistaxis in two generations; the condition was made a distinct clinical entity by Rendu.

II, 1, aged 52 years, had had severe daily recurring epistaxis for three weeks. His father (I, 1) had died of dysentery, with repeated crises of melena, at the age of 55, and his mother (II, 2) was subject to recurring epistaxis. One brother (II, 2) had also suffered from abundant and recurring epistaxis. Telangiectases were noted on the skin of the face and mucous membranes of the mouth and tongue of the patient. They were also seen, but in less numbers, over the neck and chest.

FAMILY 5 (Fig. 10) (Osler, 1901).—There were attacks of epistaxis from childhood, seven members of the family being subject to it; there were telangiectases on skin of face and mucous membranes of nose and mouth.

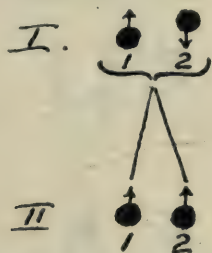


Fig. 9.—Family 4 (Rendu).

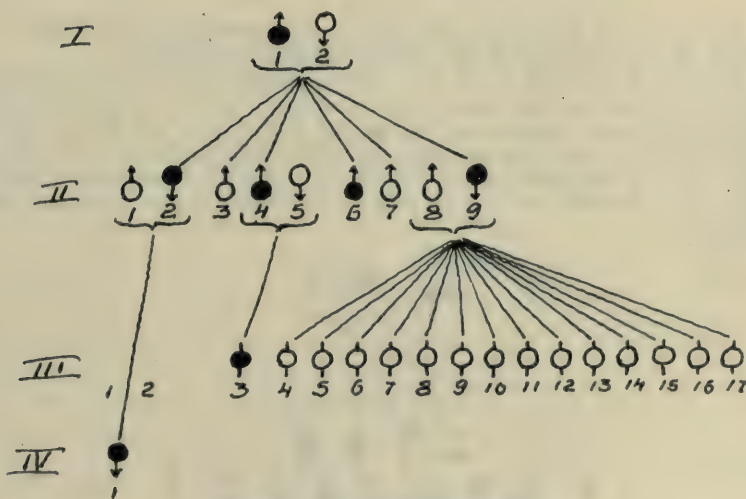


Fig. 10.—Family 5 (Osler 1).

II, 4 began to have attacks of epistaxis in his 10th year. They were not very severe, but recurred almost every day until he was 37 years old, when he was unable to work for nearly three years on account of the weakness and anemia induced by the bleeding. He was 49 years old when first seen by Osler, and exhibited telangiectases on the ears, nose, cheeks, lips and tongue. His father (I, 1) had had attacks of epistaxis, which were never dangerous but very frequent. His oldest sister (II, 2) had bled from childhood from the nose and her granddaughter (IV, 1) had had epistaxis frequently. Another sister (II, 9), also, had bled from the nose and mouth since childhood. His child (III, 3) had likewise occasionally bled from the nose, while a brother

(II, 6) gave a history of epistaxis from childhood with telangiectases of the skin and mucous membranes, which bled at times. This last patient finally died of cancer of the stomach, as revealed by necropsy.

FAMILY 6 (Fig. 11) (Osler, 1901).—Recurring epistaxis had occurred in the members of the family from the 10th year; there were multiple telangiectases of the skin and mucous membranes of the nose and mouth, and telangiectases but no epistaxis in the next generation.

Patient I, 1, aged 49 years, had had epistaxis from his 10th year, varying greatly in severity. From his 18th to his 21st year he was much better, but then the attacks came on again with increased severity. When a boy he first noted the telangiectases, which have increased during the past seven or eight years and are situated on his ears, forehead, cheeks, lips, back, chest, abdomen and hands. One of his sons showed vascular nevi (II, 1).

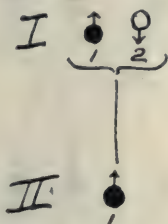


Fig. 11.—Family 6 (Osler 2).

FAMILY 7 (Fig. 12) (Josserand, 1902).—Telangiectases and epistaxis were observed in four members of the family during two generations.

Patient II, 1, aged 56 years, had suffered from epistaxis from childhood. Of late years she had had hemorrhages also from the lips, gums and tongue. Telangiectases were observed on her cheeks, lips, tongue and palate and less markedly on her neck, breast, back and arms. Her father (I, 1) and one brother (II, 2) had suffered from recurrent epistaxis from early childhood, while another brother (II, 3), in addition to this symptom, presented facial telangiectases.

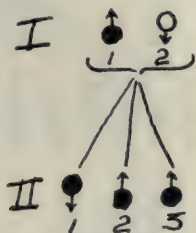


Fig. 12.—Family 7 (Josserand).

FAMILY 8 (Fig. 13) (Brown-Kelly, 1906).—There was severe recurring epistaxis with multiple telangiectases of the skin and mucous membranes in the family for three generations; death of one member occurred from syncope induced by a prolonged epistaxis.

The patient, II, 2, aged 41 years, had suffered from severe recurring epistaxis. Her father (I, 1) had died at 62 years of age from the effects of frequent attacks of epistaxis. A sister (II, 3) has suffered from frequent hemorrhages from her lips, mouth and nose, and has multiple telangiectases on the skin of her face and hands, and the mucous membranes of her nose and mouth. A few have lately developed on the scalp and occasionally give rise to

bleeding when the hair is combed. Her daughter (III, 1) bleeds from her nose and has red spots on her face. The patient (II, 2) finally died from an attack of syncope induced by severe and persistent epistaxis.

FAMILY 9 (Fig. 14) (Hawthorne, 1906).—There was epistaxis in three generations, with telangiectases, a marked family history of these symptoms.

II, 3, aged 49 years, had suffered from childhood with bleeding at the nose, which once required plugging of the nostrils. Her father (I, 1) and her sister (II, 1) had similar attacks and all had telangiectases on their faces.

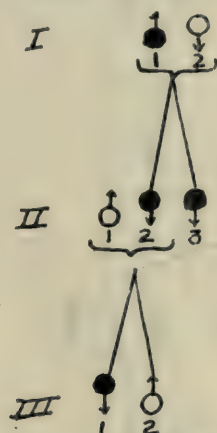


Fig. 13.—Family 8 (Brown and Kelly).

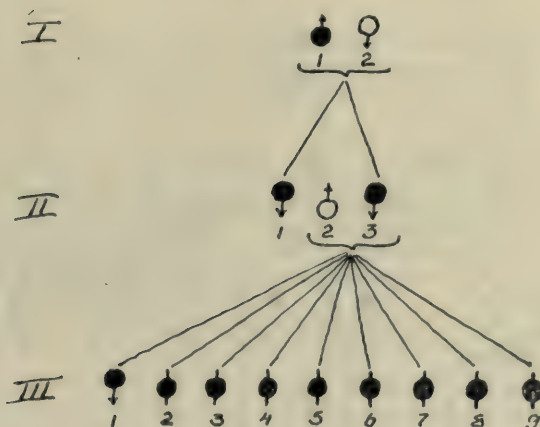


Fig. 14.—Family 9 (Hawthorne).

A few were also seen on the patient's fingers (II, 3) and all of her nine children were subject to recurring nose bleed.

FAMILY 10 (Fig. 15) (Osler, 1907).—Hemorrhages occurred from nose and mouth of one member of the family from the 10th year; there were multiple telangiectases on the skin of his face, ears and lips. A similar condition was observed in three generations.

The patient, III, 1, aged 53 years, is a physician, whose grandfather (I, 1), father (II, 1) and one sister (III, 3) had red spots on their faces. His son



(IV, 1) is subject to attacks of epistaxis which have been frequent but not profuse. III, 1 has bled with great profuseness from his nose and from the telangiectases on his head, face, ears, arm and mucous membranes of his mouth.

FAMILY 11 (Fig. 16) (Parkes-Weber, 1907).—This family gives a history of recurring epistaxis in four generations; telangiectases also were observed in four of the eight members affected with telangiectases.

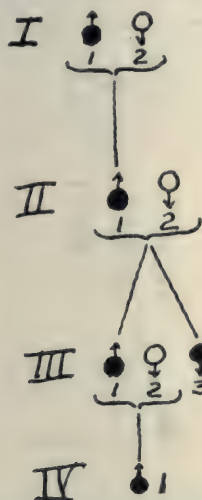


Fig. 15.—Family 10 (Osler 3).

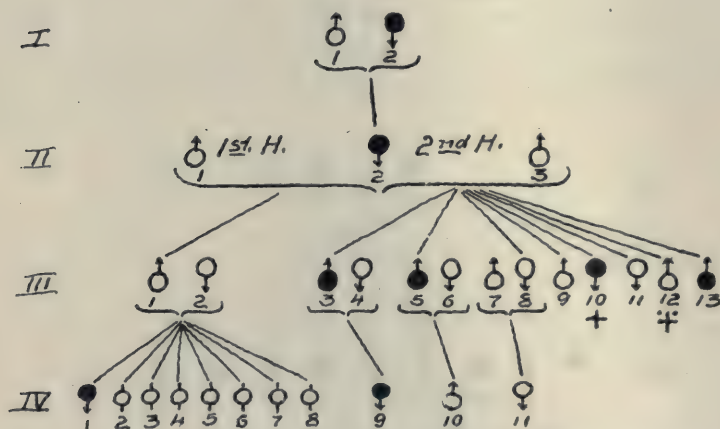


Fig. 16.—Family 11 (Parkes-Weber). The daughter, III, 10 suffered with epistaxis only. The son, III, 12, aged 3 years, died of "brain trouble."

The patient, II, 2, aged 60 years, first noted telangiectases on her face when she was 42 years old. Recurrent attacks of epistaxis had begun a few years earlier. During the last few years they have come on every two or three weeks and are more severe than formerly. The telangiectases are located on her face, ears, lips, tongue, mucous membranes of her mouth and nose and the conjunctival surface of the four eyelids. They are also observed on the

anterior surface of the epiglottis and on the fingers and under the finger nails. All are punctiform save one on the right cheek, which is of the spider nevus type. Her mother (I, 2) was subject to attacks of epistaxis and had one or two spots on her face. By her first marriage, she had a son, whose boy (IV, 1), aged 9, suffers from epistaxis. By her second marriage she has three sons (III, 3, 5 and 13) and one daughter (III, 10) who suffer likewise from epistaxis and two of these sons (III, 3 and 5) have multiple angiomas of the skin and mucous membranes. The oldest son (III, 3) is married and has a boy (IV, 9) who is subject to attacks of epistaxis.

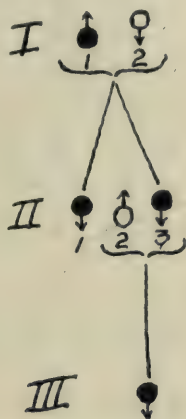


Fig. 17.—Family 12 (Phillips).

FAMILY 12 (Fig. 17) (Phillips, 1908).—Recurring hemorrhages from the nose and mouth occurred in this family in three generations; there were multiple telangiectases of the nose, tongue and buccal mucous membrane.

The patient, II, 3, aged 56 years, was subject to bleeding from childhood from the mouth and more recently from the nose. Her father (I, 1) suffers from violent epistaxis and bleeding from the tongue, while a sister (II, 1) died from hemorrhage of the gums. The patient (II, 3) has a daughter who has telangiectases on her tongue and recently has had attacks of epistaxis. The patient exhibits telangiectases on the mucous membranes of the tongue and nose, as well as on the buccal mucous membranes.

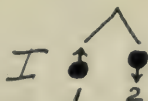


Fig. 18.—Family 13 (Waggett).

FAMILY 13 (Fig. 18) (Waggett, 1908).—There was severe epistaxis in brother and sister; multiple telangiectases of the skin of the face and the mucous membranes of the nose and mouth.

The patient, I, 1, aged 53 years, has had hemorrhage from the nose frequently since his 20th year. He has likewise had hemorrhages from telangiectases on his face and lips. These telangiectases are also seen on the mucous membranes of his tongue and nose. His sister (I, 2) has the same symptoms.

FAMILY 14 (Fig. 19) (Ballantyne, 1908).—This was a family of eight Hollanders who presented telangiectases in five of the six members examined.

The mother (I, 2) of a family of six children exhibited telangiectases on the conjunctiva of the right upper lid, the cheeks, the nose and the lips, but gave no history of hemorrhages from any of them. Her only daughter (II, 2) showed similar spots in almost similar locations, but has one also under the nail of the fourth finger of her right hand. She has bled from the nose rather frequently. A brother, II, 3, had no spots, but was subject to attacks of epi-

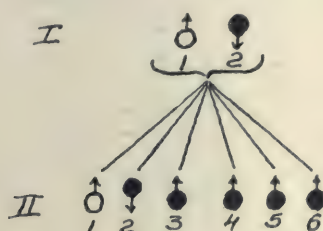


Fig. 19.—Family 14 (Ballantyne).

staxis. Another brother, II, 4, had a few telangiectases on the tip of his nose, inner surface of his lower lip and the tip of his tongue. The brothers, II, 5 and 6 showed small elevated red spots about the tip of the tongue as II, 4 did.

FAMILY 15 (Fig. 20) (Gottheil, 1908).—There were telangiectases in two generations; epistaxis in three.

The patient, II, 5, aged 40 years, has bled periodically and spontaneously from his nose, tongue and lips as long as he can remember. The hemorrhages

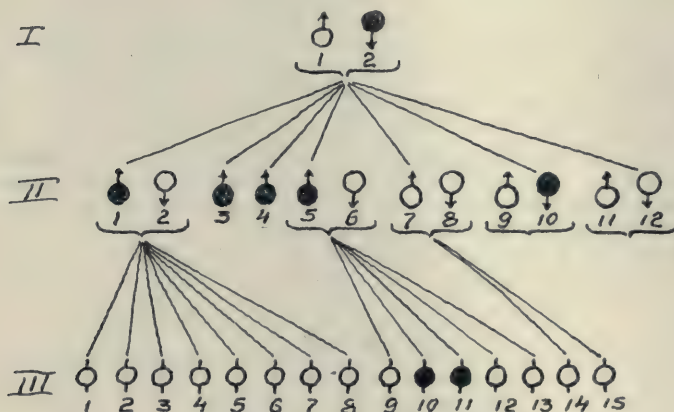


Fig. 20.—Family 15 (Gottheil).

vary in frequency from several in one day to one a week, but recently they have come on more often. His mother (I, 2) had spots on her lips and was said to have died from hemorrhages twenty-seven years ago. His brothers (II, 1, 3 and 4) have also had hemorrhages from the nose, as has also one sister (II, 10). None of them have had telangiectases. Two of his children (III, 10 and 11) have bled from the nose also.



FAMILY 16 (Fig. 21) (Hanes, 1908).—There were recurring hemorrhages in four generations of one family, and multiple telangiectases of skin and mucous membranes, but no symptoms of hemophilia.

The patient, II, 4, aged 53 years, had been subject to epistaxis from childhood. Generally her nose bleeds once or twice a week and sometimes every day. Since her 37th year the epistaxis has increased in severity, as well as the telangiectatic spots on her face, lips and tongue. Some are also seen on the ears, the hard palate and the gums of the lower jaw, as well as on the nasal mucous membranes. On the pads of the fingers and under the nails many small purple spots are visible. The conjunctiva of the right lower lid exhibits one of bright red hue. Her mother (I, 2), who died at 48 from heart disease with dropsy and cough, had suffered all her life with severe epistaxis. Her brothers (II, 1 and 2) were also troubled all their lives with a similar severe epistaxis and both showed red spots on their face and lips. One son (III, 1) has been greatly troubled from severe hemorrhages from telangiectases, which

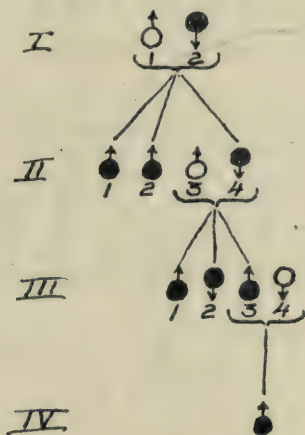


Fig. 21.—Family 16 (Hanes 1).

are numerous on his lips and tongue. Frequently he injures one of these labial or lingual telangiectases while eating, so that blood will spurt from the injured spot and render further progress with the meal impossible. Profuse hemorrhages from two telangiectases under the nail of the left middle finger have interfered greatly with his occupation as a fireman. Occasionally he has attacks of epistaxis. In every instance the hemorrhages result from slight traumatism. The other son (III, 3) had spots on his lips and face and had bled easily from his nose all his life. Her grandson (IV, 1), a son of III, 3, has two small red telangiectases at the mucocutaneous junction, but has never bled from the nose. Her daughter (III, 2), on the other hand, has frequently bled from the nose most profusely and presents on her face small pinpoint telangiectases. Several of a violaceous hue are seen on the lower lip and one bright red in color is noted on the dorsum of the tongue. The mucous membranes of the nose and the pads of the fingers and under the nails are other locations where these spots are found.

FAMILY 17 (Fig. 22) (Hanes, 1908).—There was epistaxis in four sisters and multiple telangiectases affecting chiefly the mucous membranes, but no symptoms of hemophilia.

The patient, II, 4, aged 46 years, had suffered extremely throughout childhood and youth from severe epistaxis, which frequently necessitated plugging of the nostrils. She also presented telangiectases under the eyes, at the bases of the alae nasi, on the conjunctivae, both lips, tip of the tongue, hard and soft palate, and nasal mucous membrane. She later developed an endocarditis. Her father (I, 1) had suffered from youth with recurring epistaxis, which came on daily during the later years of his life. One sister (II, 7), who

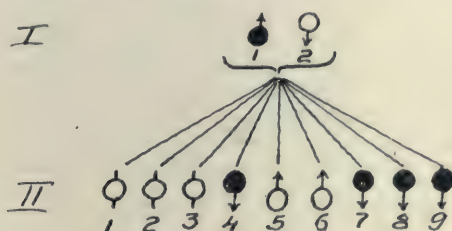


Fig. 22.—Family 17 (Hanes 2).

died at the age of 27, during childbirth, had bled profusely and frequently from the nose. Another sister (II, 8) had bled almost daily as far back as she could remember. She also presented definite telangiectases on her left cheek, lips, tip of tongue and nasal mucous membrane. Another sister (II, 9) bleeds two or three times weekly from the nose, but never very severely. She has a few telangiectases on the lips, tip of the tongue and nasal mucous membrane. Her two brothers (II, 5 and 6) are practically free from the disease, but bleed readily from the nose during a cold or after traumatism.



Fig. 23.—Family 18 (Laffont).

**FAMILY 18 (Fig. 23) (Laffont, 1909).**—A marked family history of telangiectasis, with a history of epistaxis in three out of the eight affected in the three generations.

The patient, II, 3, aged 48 years, noted eight years previously that telangiectases were appearing on the periphery of the scalp, the ear, the face, the breast and the back. Some had disappeared spontaneously. She had been subject since puberty to repeated attacks of epistaxis. Her mother (I, 2) had had, toward her 62d year, small telangiectases appear on her ear, neck, chest and

arm. There was no history of epistaxis in this instance. Her sister (II, 4) had similar spots on her ears, nape of neck and arm, but no epistaxis. Her elder brother (II, 1) had no telangiectases, but had spat up blood for about a year from varicosities of the pharynx, while her younger brother (II, 5) had some spots on his chest and back, but no hemorrhages. Her eldest daughter (III, 1) showed some telangiectases on her face and body, and had been subject to repeated epistaxis. Another daughter (III, 2) showed spots on the dorsal aspect of the forearm, the neck and trunk, while the third daughter (III, 3) presented spots on her face, neck, breast and back.

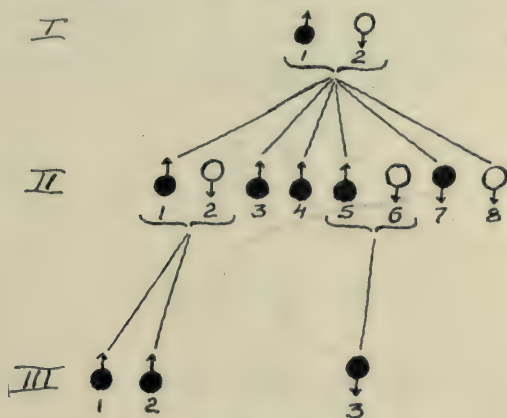


Fig. 24.—Family 19 (Langmead).

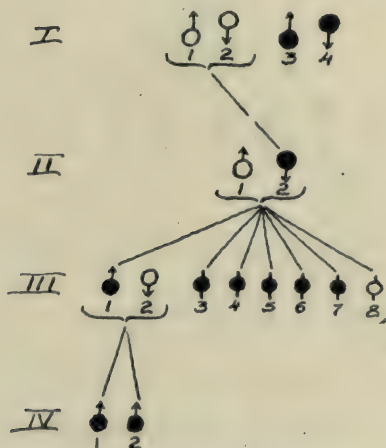


Fig. 25.—Family 20 (Audry).

FAMILY 19 (Fig. 24) (Langmead, 1909).—Telangiectases were noted in eight members of a family for three generations, epistaxis being observed in eleven instances.

The patient, II, 1, aged 68 years, was subject for about twenty years to frequent epistaxis. Occasionally telangiectases on his face and tongue have burst out bleeding. The telangiectases are seen on his face, lower lip, tip of tongue, palate and a few on the neck, chest and back. His father (I, 1) was



affected with epistaxis and telangiectasis, but his mother (I, 2) had epistaxis only. His three brothers (II, 3, 4 and 5) were subjects of epistaxis and telangiectasis, as was also one sister (II, 7). One son (III, 1) has nevoid patches and epistaxis, while the other (III, 2) has epistaxis only. A niece (III, 3) has similar patches and recurring epistaxis.

FAMILY 20 (Fig. 25) (Audry, 1911).—Epistaxis and telangiectases were noted in four generations.

The patient, III, 1, aged 70 years, had had attacks of epistaxis for many years, but lately they had increased greatly in frequency. Numerous telangiectases were seen on his face, lips, tongue and palate, and more sparsely on his trunk and upper extremity. His mother (II, 2) had epistaxis and telangiectases, as had also a great uncle and a great aunt. Five of the patient's brothers and sisters were similarly affected, as well as his two sons. Several nephews and nieces also had the same condition.

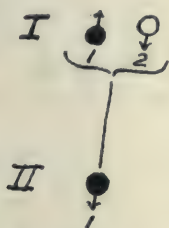


Fig. 26.—Family 21 (Osler 4).



Fig. 27.—Family 22 (Van Wagenen).

FAMILY 21 (Fig. 26) (Osler, 1911).—Telangiectases were observed in two members of a family for two generations.

The patient, II, 1, aged 35 years, had suffered from childhood from recurring epistaxis. She also had shown later some telangiectases, but they had increased considerably during the last six years. They were situated on the nasal mucous membranes, the tongue and the lips. One was also seen under the nail of the left index finger. Her father (I, 1) had suffered for many years from severe recurring epistaxis and his face was covered with telangiectases.

FAMILY 22 (Fig. 27) (Van Wagenen, 1912).—Epistaxis and telangiectases were observed in four members of a family during three generations.

The patient, II, 2, aged 32 years, has had bleeding from the tongue and nose during the past nine years. Telangiectases were seen on the nasal mucous membranes, tongue, face and arms. Her mother (I, 2) has had frequent attacks of epistaxis since early childhood and two of her brothers (II, 3 and 4) have had a similar history.

FAMILY 23 (Fig. 28) (Sequeira, 1913).—Epistaxis and telangiectases were observed twice in two generations.

The patient, I, 2, aged 55 years, has noted red spots appearing on her face and fingers during the past five or six years. Occasionally she has had

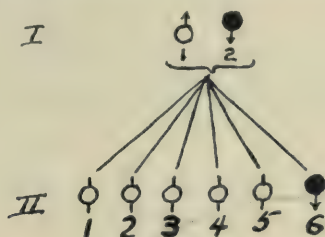


Fig. 28.—Family 23 (Sequeira).

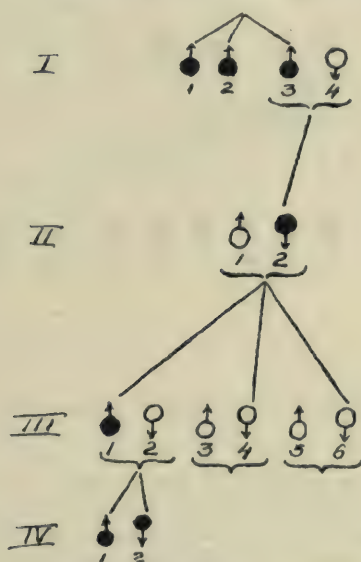


Fig. 29.—Family 24 (Gjessing).

hemorrhages from these spots. Telangiectases were also found on the tongue, mucous membrane of the lower lip, hard palate, uvula and the lower internal surface of the left labium majus. She had had also slight bleeding from the nose. One daughter (II, 6) gave a history of occasional attacks of epistaxis.

FAMILY 24 (Fig. 29) (Gjessing, 1915).—Epistaxis and telangiectases were observed for four generations, one member being affected also with endocarditis and hemorrhagic retinitis.

The patient, III, 1, 51 years of age, has had severe epistaxis from childhood, which lately has increased in frequency and violence. In his late twen-

ties telangiectases began to develop on his face and the mucous membranes of his mouth. They are now also seen on the ears, under the chin and on the neck, as well as on the nose, the tip and base of the tongue, the hard palate and the lower right eyelid. On the left arm two small ones are also noted, others under two finger nails and still others on the dorsal and volar aspects of the fingers. Besides the hemorrhages coming from the nose, bleeding has been observed from spots on his cheeks, tongue and eyelid. His maternal grandfather (I, 3) and the latter's two brothers (I, 1 and 2) had suffered from severe epistaxis, but it was not known whether they had facial angiomas. His two children (IV, 1 and 2) have suffered since childhood from severe epistaxis. The son (IV, 1) has telangiectases on his face, nasal mucous membranes, tongue, and hard palate. He is affected with a disease of the heart and has hemorrhagic retinitis. He has become so anemic from the hemorrhages that his hemoglobin has fallen to 25 (Tallqvist). The daughter (IV, 2) has a few small angiomas on her cheeks, which have appeared during the last two years.

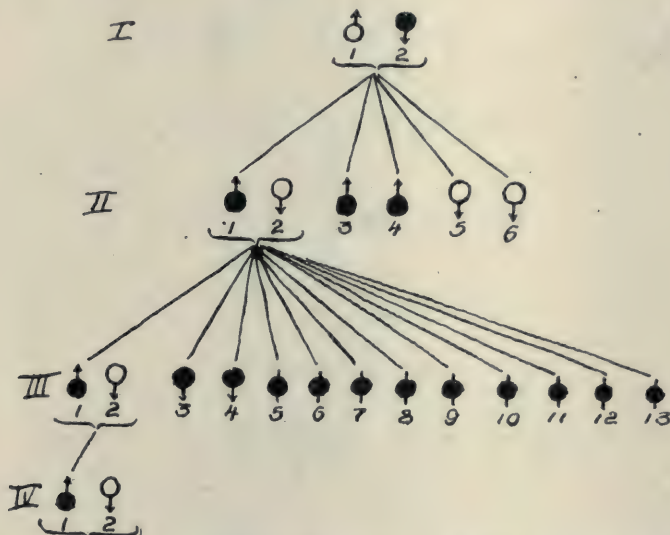


Fig. 30.—Family 25 (Hutchison and Oliver). Patients III, 5 to 13, inclusive, suffered with epistaxis only.

FAMILY 25 (Fig. 30) (Hutchison and Oliver, 1916).—There was a history of epistaxis in four generations, with telangiectases observed in three generations.

The patient, III, 1, aged 48, had had attacks of epistaxis for as long as he could remember, but during the last two years they had become more severe, especially in the morning. Telangiectases appeared first eight years ago and gradually increased in number. They were situated on the ears, cheeks, nose, lips, buccal mucous membranes, hard palate, tip and under surface of the tongue, the posterior pharyngeal wall, the soft palate, the uvula and the right hand. Spontaneous bleeding had occurred from those on the lips, and this was sometimes preceded by a feeling of soreness. He had also bled occasionally from the ears, about the fossa of the helix and from the corner of the right eye, as well as from the gums and the inside of the mouth. From the last situation the hemorrhages appeared especially after very hot drinks. About a year ago he bled from the rectum, on straining. His Wassermann reaction was negative. His father (II, 1) had had frequent spontaneous attacks of epistaxis from childhood and for the last six years has bled occasionally and



spontaneously from the spots on his nose. During the last five or six months he has bled somewhat from his gums. Two or three years ago he also had occasional bleeding from the rectum. For many years he has had telangiectases on his face. They were also noted on his ears, cheeks, sides of nose, lips, dorsum of tongue, hard palate and buccal mucous membranes, back and shoulders. His son (IV, 1) has had attacks of epistaxis as long as he could remember, but during the last two years they have become more infrequent. Some telangiectases were noted on his right cheek, the tip of his tongue, the mucous membranes of his lips and on his right shoulder. There was a definite history of nose bleeding in other members of the family (I, 2, II, 3 and 4, III, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13).

#### CONCLUSIONS

From a consideration of the histories of these three families affected with hemorrhagic telangiectasis and those previously recorded we may conclude that heredity is the only important etiologic factor as yet discovered and that both sexes are equally affected and equally capable of transmitting the disease. Microscopically a developmental defect has been found in the dilated capillaries, as the elastic and muscle fibers appear to be wanting. The capillaries, consequently, are very liable to produce hemorrhages by their rupture, which is induced either spontaneously or by traumatism. The hemorrhages from the nose are generally seen early in life, while the telangiectases are a later development. The cautery in most instances offers the best results in the treatment of the bleeding telangiectases.

# A CLINICAL STUDY OF THE SECRETIONS ON THE PROXIMAL AND DISTAL SIDES OF THE PYLORUS \*

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The secretions on the proximal and distal sides of the pylorus have been studied in 125 patients entering Lakeside Hospital during the past year, complaining of various gastro-intestinal disturbances. The secretions were obtained on a fasting stomach in all cases and in many of the cases the secretory and motor function of the stomach was also determined.

The Einhorn and Rehfuess tubes were used. The Rehfuess tip, being oval, with slits on the side, and relatively large, when passing through the pylorus, became plugged by the walls of the pylorus and no secretion could be aspirated during this time. To remedy this a small opening was made in the end.

The tube was passed, as a rule, on a fifteen-hour fasting stomach. The tip was introduced into the mouth when the patient was lying down. The patient then swallowed and as soon as the tip passed into the esophagus he assumed the sitting posture, being requested not to swallow any more and to expectorate all saliva. By supporting the tube and having the patient breathe slowly and deeply, the tip slowly passes down the esophagus into the stomach. The tubes are marked at 35 cm., 50 cm., 60 cm., 70 cm., etc., from the tip. When the 35 cm. mark passes the teeth, the tip is at the cardiac orifice. The patients were then placed on their right side. The tip then, together with what secretions are present, gravitates toward the pylorus. No secretions can be aspirated until both the tip and the gastric secretions reach the pylorus. The distance from the pylorus to the mouth is indicated by the second mark (50 cm.) on the tube. Specimens were then obtained every ten minutes until the tip passed through the pylorus into the duodenum. This took, in normal cases, about thirty minutes.

Three methods were used in obtaining the specimens:

First, the secretion was aspirated with a 20 c.c. glass syringe connected directly to the tube. It was difficult not to apply too much suction, which drew the mucosa into the openings of the tip, ruptured capillaries and almost invariably gave a positive guaiac test.

Second, in most cases sufficient secretion could be obtained by

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\* From the Medical Clinic of Lakeside Hospital, Cleveland.

siphoning or by the gravity method. The secretions came at irregular intervals. Here the chance of accidental hemorrhage is ruled out. The amount of secretion present at any given time in the stomach and the time it left the stomach cannot be accurately determined.

Third, in this method the tube was connected to a Wolff bottle. Small test tubes were hung by a thread in the bottle under the cork. Then a vacuum was made in the bottle by a Potain pump, and the contents of the stomach or duodenum were aspirated. When the test tube became full it was replaced by another, or the secretions were collected in the bottle. In this method uniform suction is obtained, all the secretion may be aspirated and measured at any given time and individual specimens at varying periods may be obtained with ease.

In obtaining the secretions with the duodenal tube the following sources of error must be considered: The stimulation of the tube in the mouth, together with some nausea, stimulates the flow of saliva, which, if swallowed, being alkaline, will decrease and often completely neutralize the free hydrochloric acid. Then, too, strong suction draws the mucosa against the openings and bursts capillaries, which will give a positive guaiac test and often microscopic blood is seen.

The average gastric secretion of the fasting stomach was found to be a clear, opalescent fluid. About 10 c.c. could be aspirated every ten minutes. A small amount of mucus was invariably present. In the same individual the acidity of the fasting stomach was found to be about one half that of the acidity after an Ewald test meal. Microscopic examination showed small shreds of mucus. A few white blood cells, usually clumped in mucus, were found, and some degenerated, squamous epithelium and bacteria of varying types.

The normal duodenal secretion is of a clear amber color. About 15 c.c. can usually be aspirated and then about 10 c.c. every ten minutes. There is no free hydrochloric acid present, the total acidity being about 5 per cent. lower than that of the gastric secretions. It remains of an amber color for several days, gradually changing to green as the bilirubin becomes oxidized to biliverdin. Microscopic examination shows fewer white blood cells, epithelium and bacteria. A large diplococcus was the commonest type seen.

When free hydrochloric acid is present, the bile pigments are precipitated. Instead of a clear, amber fluid, it is of a cloudy yellow, and a sediment settles on standing. It becomes green very rapidly, depending on the amount of free hydrochloric acid. Microscopically a fine yellow sediment is seen, which resembles amorphous urates. On neutralizing the free hydrochloric acid with sodium hydroxid the fluid becomes of a clear amber color.

The secretory function was determined in most cases by giving the patients an Ewald test meal. The Rehfuß tube was then passed and



specimens obtained every fifteen minutes until the stomach was empty. In this way the acidity was determined during the whole process of digestion, and not at just one point; and the time required to empty the stomach was also determined. The specimens obtained were examined for free hydrochloric acid, total acidity, lactic acid, blood and bile. The amount of mucus was noted. A microscopic examination was made for red blood cells, white blood cells, epithelium, bacteria and gallstone detritus. In cases of achylia or subacidity the Wolff-Junghan's test was made. The gastric motor function was determined by passing a stomach tube three hours after a house meal, and by the Roentgen ray.

The secretions on the proximal and distal sides of the pylorus were studied in the cases shown in Table 1. From the number and variety of cases only the following conditions will be considered: gastric neurosis, gastric ulcer, gastric carcinoma, duodenal ulcer, obstructive jaundice, cholelithiasis, cholecystitis and pernicious anemia.

TABLE 1.—DISEASES IN WHICH PYLORIC CONTENTS WERE STUDIED

Disease	No. Cases	Disease	No. Cases
Pernicious anemia.....	6	Cholelithiasis.....	10
Gastric ulcer.....	18	Cholecystitis.....	3
Duodenal ulcer.....	5	Gastric purpura.....	1
Gastric carcinoma.....	12	Cerebrospinal syphilis.....	3
Gastric neurosis.....	36	Nephritis.....	3
Neurasthenia.....	5	Vomiting of pregnancy.....	2
Chronic gastritis.....	5	Tuberculosis.....	2
Chronic constipation.....	10	Epigastric hernia.....	1
Obstructive jaundice.....	3		

#### GASTRIC NEUROSIS

Rehfuss<sup>1</sup> and others have shown that a very high acidity may be present in apparently healthy persons and cause no discomfort. That some have pain and discomfort with a degree of acidity that in others causes no distress we in our ignorance attribute to a neurosis, in which the threshold of excitability is low.

In 40 per cent. of the patients entering Lakeside Hospital during the past year, complaining of various gastro-intestinal symptoms, nothing pathologic could be found in the gastric secretions to account for the distress except an increase in acidity above that considered as the average. These cases were diagnosed gastric neurosis and fell into

1. Rehfuss, Bergein and Hawk: Jour. Am. Med. Assn., 1914, **63**, 909.

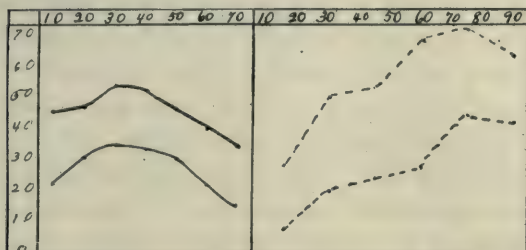


Fig. 1.—Acidity curves for cases of gastric neurosis. The solid lines indicate the average acidity of the secretions of the fasting stomach in forty cases. The dash line indicates the acidity of the secretions following an Ewald meal. The upper curves in each instance indicate the total acidity; the lower, the free hydrochloric acid.

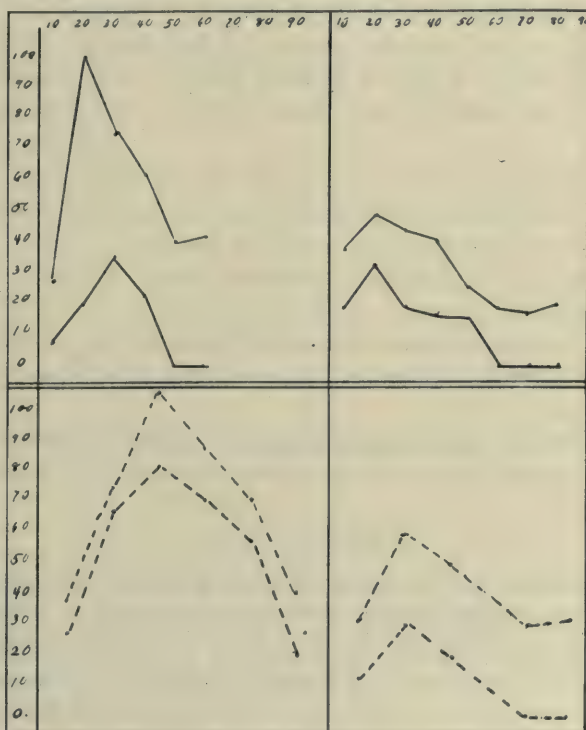


Fig. 2.—Acidity curves in four cases of gastric neurosis with chronic constipation, the upper two (Cases 77 and 98) being of the secretions of the fasting stomach, the lower two (Cases 34 and 58) of the secretions of the stomach after an Ewald meal. The upper curve in each of the four cases indicates the total acidity; the lower, the free hydrochloric acid.

three general classes: (1) gastric neurosis secondary to infection, as tuberculosis, influenza, syphilis and pelvic inflammatory conditions; (2) gastric neurosis associated with chronic constipation; (3) gastric neurosis secondary to some mental or physical strain.

The symptoms complained of were many and varied. The commonest were epigastric distress after meals, flatulence, anorexia, heartburn, nausea and pain. The pain varied in the same individual in time, location, intensity and duration. There was invariably an absence of tenderness and muscle rigidity. In the cases in which the secretions were examined more than once, they showed a marked variation in the acidity. The average acidity curve made from forty cases of the secretions of the fasting stomach, given in Figure 1, shows a constant relation of the free hydrochloric acid to the total acidity, a slight rise in acidity for the first half hour and then a gradual fall. The duodenum was entered on an average in forty minutes. The individual curves varied greatly, no two being alike. Figure 2 shows curves in four cases of gastric neurosis associated with chronic constipation, two on a fasting stomach and two after an Ewald meal. These show the futility of comparing the acidity curves in different individuals.

To summarize the results of the investigations in cases of gastric neurosis, I would say that in 40 per cent. of cases examined nothing pathologic could be found to account for distress.

A diagnosis of a pathologic condition cannot be made from subjective symptoms.

Gastric neurosis is most commonly secondary to an infection, as tuberculosis, syphilis, influenza, chronic constipation and mental or physical strain.

The secretions on the proximal and distal sides of the pylorus and the gastric acidity curves show nothing constant or typical of this condition.

#### GASTRIC CARCINOMA

The secretions on the proximal side of the pylorus were examined in eleven cases of carcinoma of the stomach, liver or colon. The secretions on the distal side were obtained in only three cases (probably because the carcinoma involved the pylorus). Two patients had carcinoma of the liver and one had carcinoma of the hepatic flexure of the colon.

In all the cases of gastric carcinoma there was no free hydrochloric acid, with one exception. In two cases of carcinoma of the liver and stomach free hydrochloric acid was present. One of these cases was found at necropsy to be complicated with a duodenal ulcer. Lactic acid was present in only three of the cases. There was an increased



bacterial flora in all specimens examined. The Wolff-Junghan's test was done in only the last three cases. It was positive in two cases of gastric carcinoma, and only faintly positive in a case of carcinoma of the splenic flexure of the colon.

The case of carcinoma of the pylorus had a very atypical clinical course. The patient was a Jew, aged 45. He entered the hospital April 9, 1915, complaining of pain between meals, eructation of gas and a bitter fluid. House meal, removed in three hours, showed 200 c.c. of a greenish, sour-smelling liquid, with free hydrochloric acid 40, total acidity 100. The benzidin test

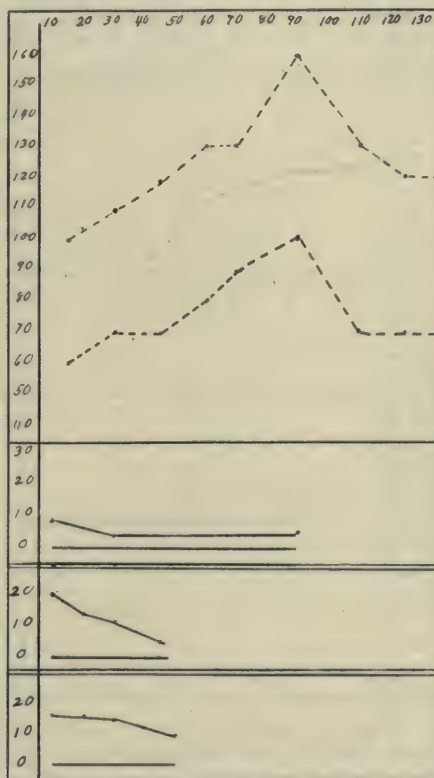


Fig. 3.—Acidity curves in cases of carcinoma of the stomach (Cases 120, 49, 83 and 68). In Case 120 each of the two dash lines indicates the acidity of the secretions following an Ewald meal. The solid lines in the other three cases indicate the acidity of the secretions of the fasting stomach. The upper curve of the four cases indicates the total acidity; the lower the free hydrochloric acid.

was positive. A carbohydrate test meal (Ewald) was given, and 30 c.c. were removed in one hour. There was free hydrochloric acid 30, with total acidity 50. No treatment was given the patient here. He had no discomfort until Oct. 1, 1915, when he began again to have pain after eating, and he became constipated. Oct. 17, 1915, he had a sharp pain in the epigastrium, followed by a sensation of weakness. He came to the hospital Oct. 18, 1915. The right side of his epigastrium was tender and rigid. A house meal was given and

200 c.c. were removed in three hours, showing free hydrochloric acid 74, total acidity 124. A carbohydrate meal was given and 75 c.c. were removed in one hour, showing free hydrochloric acid 64, total acidity 86, lactic acid negative, and guaiac test negative. An Einhorn duodenal tube was passed on a fifteen-hour fasting stomach; 150 c.c. of a coffee ground fluid were removed. The guaiac test was positive. The spectroscope showed the bands of oxyhemoglobin. Microscopic examination showed starch cells, red blood corpuscles and desquamated epithelium. The first specimen showed free hydrochloric acid 34, total acidity 50; the second, taken in forty-five minutes, showed free

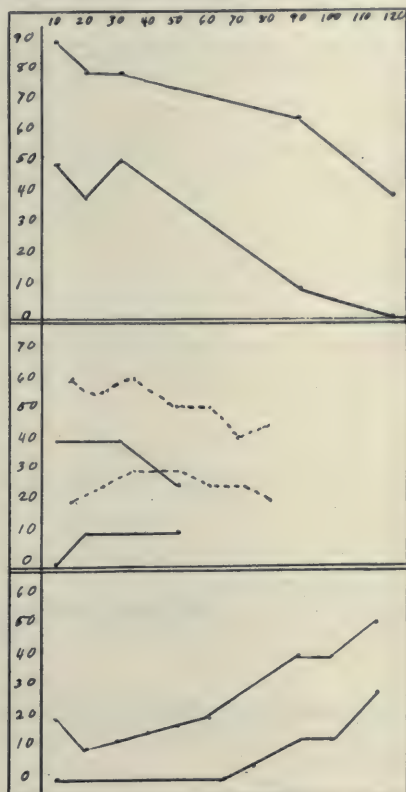


Fig. 4.—The upper part of the figure represents the acidity curves of a case of carcinoma of the liver (Case 46); the middle, of a case of carcinoma of the liver and stomach, with duodenal ulcer (Case 60); and the lower, of a case of carcinoma of the colon (Case 53). The solid lines in each case represent the acidity of the secretions of the fasting stomach; the dash lines the acidity of the secretions following an Ewald meal. The upper curve in each case represents total acidity; the lower, free hydrochloric acid.

hydrochloric acid 66, total acidity 110; and the third, taken in sixty minutes, showed free hydrochloric acid 70, and total acidity 128. The duodenum was not entered.

A diagnosis was made of *ulcus ventriculi*. The patient was given the Sippy routine. He gained in weight, got immediate relief from the pain and eructations, and when discharged, Nov. 13, 1915, he was eating a liberal diet with no discomfort. He had no further discomfort until the middle of

March, 1916. He then began to belch gas and experience heartburn. There was no pain, tenderness or vomiting. This became progressively worse, so he came to the hospital again March 29, 1916. Examination showed the stomach to be dilated. There was visible peristalsis, and a firm, movable mass was palpable to the right of the umbilicus. A duodenal tube was passed on a twenty-hour fasting stomach. Food particles were present. The duodenum was not entered. The acidity curve is plotted in Figure 3 (Case 120). The Roentgen ray showed obstruction at the pylorus, ptosis of the stomach and very little peristalsis. The patient was again put on the Sippy treatment, to see whether the retention was due to an organic obstruction or to irritation of the mucosa at the pylorus. In two weeks the Roentgen ray showed as much retention. Six hundred c.c. were removed three hours after a house meal, with free hydrochloric acid 60, total acidity 100. He was transferred

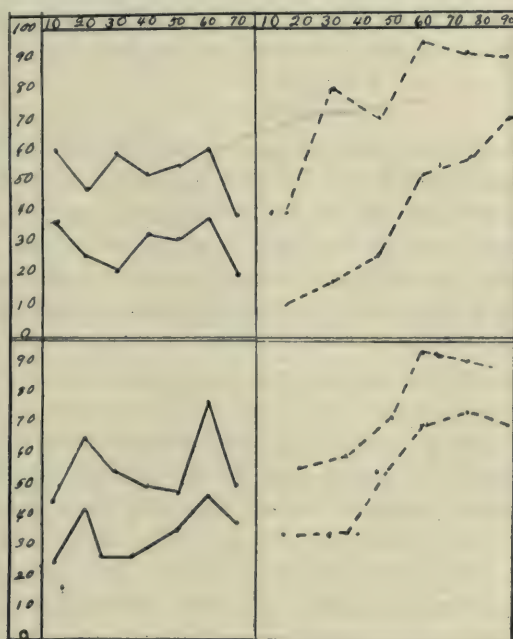


Fig. 5.—Average acidity in sixteen cases of gastric ulcer, the solid lines indicating the acidity of the secretions of the fasting stomach; the dash lines, the acidity after an Ewald meal. The upper curve in each case indicates total acidity; the lower, the free hydrochloric acid.

to the surgical service. Operation revealed a hard mass at the pylorus, and the surrounding lymph glands were hard; section of one showed a carcinoma simplex. The interesting features here are the typical signs and symptoms of a gastric ulcer, followed by retention, the presence of carcinoma four months later with a metastasis into the lymph glands and hyperacidity. A posterior gastro-enterostomy was done. The mass at the pylorus will be removed at a later date by Dr. Crile, so sections of the mass have not been seen.

The question arises: Did the patient have a gastric ulcer and the carcinoma develop in the past four months, or were the symptoms all due to carcinoma? This cannot be answered.



The acidity curve was plotted in eight of the cases in which sufficient specimens were obtained. In four cases of gastric carcinoma the curves show no free hydrochloric acid. The total acidity either remains constant or diminishes. In the one case above described the curve shows a sharp rise.

In carcinoma of the liver free hydrochloric acid was present (Fig. 4). In both cases the acidity curve descends. In the case of carcinoma at the hepatic flexure of the colon, there was a primary fall and then a late rise (Fig. 4). The same type of curve was obtained in cases of pernicious anemia, with the exception that there was no decrease in the total acidity, which remained practically constant.

In general, then, we can say that in achlorhydria, simple and malignant, the acidity curve has a tendency to descend.

#### GASTRIC ULCER

There were eighteen cases of gastric ulcer in this series, in which a positive diagnosis could be made. The diagnoses were made from the history of hematemesis and blood in the gastric secretions, or by the Roentgen ray in cases of old chronic ulcers or scars. In these few cases it was seen that the pain varied greatly in location and time of occurrence. It is of no more value diagnostically than that found in gastric neurosis.

The tenderness and rigidity were present in over 90 per cent. of cases, whereas pressure usually gave relief in cases of gastric neurosis. The nausea, vomiting and hematemesis were present in most cases.

The duodenal tube passed through the pylorus in only two cases. One case had an old callous ulcer on the lesser curvature, as shown by the Roentgen ray. This was excised. The tube went through the pylorus in the other case in twelve hours. This patient was subsequently operated on, on account of severe hemorrhage, and a pyloric ulcer was found.

This patient was a woman, aged 28. In August, 1914, she began to have pain across her back. In May, 1915, she began to belch gas, which relieved the heavy feeling in her stomach. Later the gas was accompanied by bitter acid eructations, which kept increasing in amount. Sept. 18, 1915, she began to experience sensations in her abdomen resembling fetal movements. Sept. 20, 1915, she started to vomit, at intervals from three to four days, generally four hours after eating. On one occasion she noticed food present in the vomitus which she had eaten twenty-four hours previously. She lost 20 pounds in three months. She entered the hospital Dec. 8, 1915.

Physical examination showed the stomach was distended. There was visible peristalsis and a succussion. There was muscular rigidity and tenderness in the epigastrium. Just below the costal margin in the right nipple line a firm mass, about the size of a walnut, was palpable. A stomach tube was passed and 48 ounces of fluid were removed. This showed free hydrochloric acid 20, total acidity 48. The guaiac test was negative. Cranberry seeds were present which she had eaten forty-eight hours before. From the marked obstruction,

with practically no pain preceding it, and a history of syphilis in the husband, the possibility of the obstruction being due to a gumma was considered, although the Wassermann reaction on the blood was negative. She was given anti-syphilitic treatment and put on a soft diet. She gained 18 pounds in twenty-two days. The peristalsis became less, the stomach showed less retention, and the patient was more comfortable. On Jan. 9, 1916, she vomited, felt very weak and had several black, tarry stools. The hemorrhage continued until Jan. 11, 1916. Her hemoglobin was then 40.1, red blood cells 1,424,500. She was transfused with blood, and a laparotomy was done. A large ulcer was found at the pylorus. Sutures were passed through the mass to stop bleeding and a posterior gastro-enterostomy done. The patient recovered.

In one other case there was a severe hemorrhage. The hemoglobin was 40, red blood cells 800,000. She was transfused and then given the Sippy diet without alkalies, there being a hypoacidity. This patient is gaining in weight and has no gastric distress. Operation will not be recommended. The Wolff-Junghan test is negative.

Three of the patients with gastric ulcer came in for diagnosis. No treatment was given them here.

The other cases, twelve in number, were given modifications of the Sippy routine. In most cases there was relief from nausea, vomiting and pain on the second day. About the second week their appetite returned. The majority gained in weight. In comparison with the Lenhardtz routine, the patients on the Sippy routine got relief quicker, and gained more in weight and strength. The routine followed is given in Table 2.

TABLE 2.—DIET IN THE MODIFIED SIPPY TREATMENT \*

Days	Milk and Cream, Ounces of Each, 6 a.m. to 8 p.m.	Soft Eggs		Cereal and Sugar, Ounces	Cream Soups, Ounces	Jellies, Ounces	Stewed Fruit, Ounces	Toast
		A. M.	P. M.					
1	½ per hr.							
2	1 per hr.							
3	1½ per hr.							
4	1½ per hr.	1	1					
5	1½ per hr.	2	2					
6	1½ per hr.	2	2	3 noon				
7	1½ per hr.	2	2	3 twice a day				
7 to 14	1½ every 2 hr.	2	2	3 twice a day	3			
14 to 21	1½ every 2 hr.	2	2	3 twice a day	3	3	2	
21 to 28	1½ every 2 hr.	2	2	3 twice a day	3	3	3	Twice a day

Before discharging the patients, they were given a liberal diet, with no alkalies. A test meal was then obtained. If they had a return of their gastric symptoms, or if the gastric analysis showed a hyperacidity, they were advised to continue taking alkalies and a suitable diet was suggested.

As a modified B. W. Sippy treatment for gastric ulcer I would give the following:

1. Absolute rest in bed for three weeks, with 1 dram of bismuth subcarbonate in 3 ounces of water each morning before feedings are begun.

Midway between the feedings the two following are given alternately:

2. Ten grains each of heavy calcined magnesia and sodium bicarbonate in  $\frac{1}{2}$  ounce of water.

3. Ten grains each of bismuth subcarbonate and sodium bicarbonate in  $\frac{1}{2}$  ounce of water.

As a diet the course outlined in Table 2 is given.

#### THE RELATION OF THE ACIDITY CURVES IN THE FASTING STOMACH AND AFTER AN EWALD MEAL

The acidity curve in patients having the same pathologic conditions, such as in gastric ulcer, were compared. The comparisons showed a marked individual variation in both the fasting secretions and in those obtained after an Ewald meal.

In comparing the curves having different pathologic conditions no uniformity was found. No curve was demonstrated as typical of a gastric ulcer or of a duodenal ulcer.

By grouping all the cases and plotting a curve from the average acidity, a curve was obtained which represented the average conditions. Such a curve can only be plotted accurately with a large number of cases; for the individual variations are so great. The curves shown in Figure 5 cannot be considered as typical of gastric ulcer, etc., only the average curve of the cases examined here.

In gastric neurosis we find that the amount of free hydrochloric acid bears a constant relation to the total acidity; that there is a slight increase in acidity, due probably in the fasting stomach to the stimulation of the tube, and then a gradual fall; that the mean free hydrochloric acid is 20, total acidity 40; that after an Ewald meal there is a gradual rise in the acidity for seventy-five minutes, and then a gradual decrease. The mean acidity was free hydrochloric acid 30, total acidity 60. In gastric ulcer the mean acidity is greater, and the curve fluctuates more, probably due to a fewer number of cases from which this curve was plotted.

In duodenal ulcer the acidity is greatest, and the stomach emptied itself sooner than in other conditions. In carcinoma and pernicious anemia there is a drop in acidity after passing the tube, both in the fasting secretions and after an Ewald meal, or else the total acidity remained constant.

There was no constant relation in the same individual between



the acidity curve of the fasting secretions and the acidity curve of the secretions obtained after an Ewald test meal. In most cases the acidity went higher after the Ewald meal. In some cases there was a descending curve for the fasting secretions and an ascending curve after a test meal.

A summary of the conditions observed in an investigation of the relation of the acidity curves in the fasting stomach and after an Ewald meal is as follows:

1. There is no curve which represents the normal acidity.
2. There is no curve which is typical of a certain pathologic condition.
3. There is no constant relation between the acidity curve of the fasting secretions and those obtained after a test meal in the same individual.
4. The average curve in carcinoma and pernicious anemia has a tendency to descend; in other conditions to ascend. The highest ascent was in duodenal ulcer.

#### DUODENAL ULCER

A diagnosis of duodenal ulcer was made only when no blood was found on the proximal side of the pylorus, and was found on the distal side, and in which the stool showed a positive guaiac on a benzidin-free diet. Roentgen ray was also used in most cases and showed a filling defect of the duodenal cap. There were only five such cases. From the subjective symptoms in other cases, the presence of a duodenal ulcer was suspected, but in the absence of any physical signs such a diagnosis was not made.

The gastric secretion of the fasting stomach showed, as a rule, a greater acidity than was found in other conditions. The color was not abnormal. The duodenal secretions, in addition to the presence of blood, contained an increased amount of exfoliated epithelium cells and white blood cells.

#### OBSTRUCTIVE JAUNDICE

Prior to the use of the duodenal tube here, a diagnosis of complete obstruction of the common bile duct was made when the patient was jaundiced, the urine contained bile pigments and no urobilin, and the stool did not show the presence of bile pigment or urobilin. Since using the duodenal tube here several such cases have been examined and all showed that bile was entering the duodenum, with the exception of two cases, in which no bile could be demonstrated.

The first case was in a woman, aged 56. Her health was good previous to October, 1913. At that time she began to have epigastric distress, flatulence, heartburn and occasional attacks of vomiting. January, 1915, she began to have cramp-like pains in the left hypochondrium, which radiated to the left

breast and shoulder. From then on she became jaundiced. She entered the hospital Sept. 3, 1915. Physical examination showed tenderness and rigidity over the region of the gallbladder. The liver edge was palpable 2 cm. below the costal margin. She was deeply jaundiced. A diagnosis of cholelithiasis was made, and on September 9 a laparotomy was performed. The gallbladder was distended and contained numerous small stones. It could not be emptied by pressure, although no stones could be palpated in the common duct. A cholecystostomy was done, and the stones were removed. The obstruction of the common duct could not be cleared without prolonging the operation beyond the limit of safety.

On October 7, one month after the operation, the patient's jaundice had decreased, the stool was clay-colored, the mercury bichlorid test for bile pigment was negative, and there was no urobilin in the stool. Hemoglobin was 65 per cent., red blood cells 3,400,000. The patient was much weaker than she was before the operation. The sinus into the gallbladder was still draining. The urine contained bile pigments and salts. The total nitrogen was 0.119 gm. per 100 c.c., and the ammonia nitrogen 0.01576 gm. per 100 c.c. The ammonia nitrogen was 1 per cent. The blood plasma also contained a large amount of bile pigment and bile salts.

An Einhorn duodenal tube was passed on a fifteen-hour fasting stomach. The secretion obtained on the proximal side of the pylorus was a clear, opalescent liquid, with a trace of mucus. There were no bile, and no blood. There was free hydrochloric acid 8, with a total acidity of 22. In one and one-quarter hours the secretion obtained had the same characteristics, with free hydrochloric acid 8, and total acidity 20. The bile test was doubtful. A quantitative test for trypsin showed a normal amount, thus proving that the obstruction was not at the papilla of Vater. The duodenal tube was left in for ten days, during which time 1 ounce of her own bile was collected from the incision, and fed through the tube with 6 ounces of milk and one egg, three times a day. In addition the patient ate a soft diet. At the end of this time she had gained in weight and strength. Her hemoglobin had come from 65 to 75 per cent. and her red blood cells were 4,672,000. Yet in spite of the fact that she had been getting 3 ounces of her bile every day, the stool remained absolutely acholic and no urobilin could be demonstrated. A cholecystenterostomy was then made. The patient made a complete recovery.

The only other case which showed a complete obstruction of the common duct was in a young man, whose only complaint was jaundice and malaise. No bile could be demonstrated in his duodenal secretions for three days. On the third day bile was obtained, which contained colon bacilli. On that day he felt better. The icterus began to disappear, the bile pigments and salts diminished in his urine, the stool contained bile pigments and in four days the amount of urobilin in the stool had increased. A diagnosis of catarrhal jaundice was made.

Three other cases showed all the signs of a complete obstruction of the common duct, namely, choluria, cholemia, icterus and acholic stools, except that the duodenal secretions contained bile. One was diagnosed syphilitic and alcoholic hepatitis. The secretions on the distal side of the pylorus were normal in color and acidity, but decreased in amount. Only 4 c.c. were obtained after aspirating for fifteen minutes. With antisyphilitic treatment the liver edge came from the level of the umbilicus to the costal margin in the nipple line, the jaundice disappeared and the urine and stool became normal.



The other cases were diagnosed cholelithiasis. One patient was operated on and a stone was found in a dilated common duct. The other patient refused operation and was discharged unimproved

#### CHOLELITHIASIS

The normal duodenal secretions contain no free hydrochloric acid. It is a clear, amber fluid, and remains amber for several days, gradually changing to a green color, as the bilirubin becomes oxidized to biliverdin. However, if hydrochloric acid is present it becomes of a cloudy yellow color, due to the precipitation of bile pigment by hydrochloric acid. A sediment forms on standing, which, microscopically, is seen as a fine, light-yellow detritus. The bilirubin becomes oxidized to biliverdin immediately after removal and often before, as evidenced by the green color.

The duodenal secretions found in four patients having more or less typical symptoms of gallstones contained a heavy sediment, which was different from that seen in other cases. It was heavier and of an orange yellow color. The duodenal secretions were cloudy. Microscopically the individual particles of detritus were larger and more highly colored than those precipitated by acid. It stained very poorly with methylene blue.

Three of these patients were operated on and the gallbladder was found to contain numerous small stones. The bile aspirated directly from the gallbladder presented the same microscopic picture. In one case there were only two or three hard stones, but a soft, putty-like detritus filled the bladder. The other patient is to be operated on this summer.

There are two possibilities as to the origin of this detritus. One is that the formed stones, by the peristalsis or contractions of the gallbladder and motion of the abdominal wall, grate against each other and thus produce the detritus. The other is that it is formed in the same manner as are the stones, the stones being larger and older. However, on finding an orange yellow, coarse detritus in the duodenal secretions, together with a history of colicky pains, or jaundice, a positive diagnosis of numerous gallstones can be made. No abnormal findings were present in the duodenal secretions in cases of cholelithiasis in which only one stone was present.

#### CHOLECYSTITIS

The normal duodenal secretion is a clear, amber-colored fluid. It becomes of a cloudy yellow color when free hydrochloric acid is present. When neutralized with sodium hydroxid it becomes clear again. An alkaline duodenal secretion that is cloudy is very suggestive of cholecystitis. The cloud is due either to bacteria or white blood cells. The addition of sodium hydroxid does not clear the fluid.



Some saliva is invariably swallowed. This saliva contains bacteria, white blood cells and desquamated epithelium. Therefore a few bacteria, pus cells and epithelium are always found in the secretions on the proximal and distal sides of the pylorus. Those elements present on the distal side of the pylorus are fewer in number, but morphologically the same as those found in gastric secretions. The first specimen removed from the stomach contains more bacteria and cellular elements than the succeeding specimens.

In inflammatory conditions of the stomach, duodenum and biliary tract, the number of white blood cells and exfoliated epithelium is increased. The epithelial cells from the stomach, duodenum and biliary tract are easily differentiated from the squamous epithelium from the mouth. They are oval and the cells are larger. McNeil<sup>2</sup> distinguishes the pus cells swallowed from those arising in the stomach or duodenum by the fact that the former are clumped in mucus, the latter are found free. McNeil also differentiates the epithelial and white blood cells of the biliary tract from those of the duodenum and stomach by the yellow color of the former. We have not made that differentiation, because, if a cover-slip preparation is made from duodenal secretions which contain pigmented epithelium and white blood cells, and then sodium hydroxid be added, while under observation the pigment dissolves out, leaving all the cells nonpigmented. Then while the preparation is still under the microscope, if dilute hydrochloric acid is added, the bile pigment is seen to precipitate and clump; some pigment is deposited on white blood cells and epithelium; and, further, in the normal clear, amber, duodenal secretion, none of the epithelial cells are stained, and some of the cells, no doubt, come from the biliary tract. The secretions on the proximal and distal sides of the pylorus were in most cases examined microscopically for bacteria. Cultures were made only when a motile bacillus was found, or when those found in the duodenum appeared different in number and type from those present in the gastric secretions. In a few cases colon bacilli were found in the duodenal secretions and not in the gastric secretions. In most cases there were no colon bacilli. An attempt was made to see if there was a constant level in the duodenum at which the colon bacilli were present. The tip was allowed to go into the duodenum the length of the tube, 90 cm. from the mouth and 30 cm. from the pylorus. Specimens were obtained every 10 cm. and cultures made. In general, colon bacilli were obtained more frequently the farther down in the duodenum the tip was. The distance from the pylorus that they appeared was not constant; the individual variations were great.

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2. McNeil, H. L.: *Am. Jour. Med. Sc.*, 1916, **151**, 906.

The typhoid bacillus was found in the duodenal secretions of one patient. He was a man about 49 years old. He entered the hospital complaining of some pain and distress in right hypochondrium. He had sensory disturbances on extremities, loss of vibratory sense and diminution of other sensations. Tenderness and rigidity in right hypochondrium. Physical examination, including the secretory and motor function of the stomach, spinal fluid and blood was negative. His duodenal secretion was slightly cloudy, the reaction faintly acid. Microscopic examination showed numerous motile bacilli, which were not present in the gastric secretions. Cultures were grown on slant agar. These were then plated. A motile organism was isolated, which caused no fermentation in dextrose, maltose, lactose or saccharose bouillon, thus proving to be the bacillus typhosus. His symptoms probably were due to a typhoid cholecystitis.

In December, 1915, a Japanese patient entered the ward with a diagnosis of bleeding duodenal ulcer. Physical examination revealed dullness at the right base. He had a very foul, putrid expectoration. Roentgen ray showed an opacity at the right base, probably a lung abscess. His abdomen was very rigid and tender, especially in the right hypochondrium. The liver edge was palpable and he was jaundiced. A large amount of dark blood was passed per rectum. This was found to be due to internal hemorrhoids. These were operated on and the hemorrhage ceased. A Rehfuß tube was passed on a fifteen-hour fasting stomach. While passing the tube the patient expectorated a large amount of yellow, fetid, purulent mucus.

TABLE 3.—FINDINGS BY MEANS OF REHFUSS TUBE IN A CASE OF GASTRITIS, DUODENITIS AND CHOLECYSTITIS

Specimen	Time, Min.	Characteristics	Mucus	Free HCl	Total Acidity	Bile	Blood
1	..	.....	Too much mucus to titrate	...	..	0	0
2	25	Cloudy yellow	+++	0.4	20	+	0
3	35	Cloudy yellow	++	0	24	++	0
4	50	Clear amber	.....	0	20	+++	0

Microscopic examination of a second specimen showed a very large number of pus cells, both free and in clumps of mucus. The secretion consisted of a large amount of mucus. The mucopurulent secretion swallowed could be differentiated from the gastric secretion by the yellow color. There was a large number of bacteria, a large diplococcus predominating. There was also an increased amount of squamous epithelial cells.

Examination of the duodenal secretion showed an increased amount of mucus, but there was no blood. Microscopic examination showed a few white blood cells, which were not clumped in mucus. There were a few oval epithelial cells. Some were stained yellow and they contained a small coccus. There were numerous extracellular organisms resembling staphylococcus.

From the increased amount of mucus, white blood cells, desquamated epithelium and bacteria in the secretions, a diagnosis of chronic gastritis was made. From the same findings in the duodenum, namely, mucus, pus cells, bacteria, exfoliated oval epithelium, a duodenitis was shown to be present. From the presence of exfoliated oval epithelial and pus cells in the duodenal secretions, the jaundice, large liver and tenderness in the right hypochondrium a diagnosis of cholecystitis was made. The gastritis, duodenitis and cholecystitis was secondary to the pulmonary infection.

As a summary of the findings in cases of cholecystitis, the following points may be mentioned:

1. The normal alkaline duodenal secretion has a clear, amber color.
2. The presence of free hydrochloric acid produces a cloudy yellow duodenal secretion, which becomes clear when neutralized.
3. An alkaline duodenal secretion that is cloudy is suggestive of an inflammatory condition in the duodenum or biliary tract. The cloud is due to the presence of bacteria and pus. It does not clear on the addition of sodium hydroxid.
4. Epithelial and white blood cells, which are stained yellow by the bile pigment, do not necessarily come from the biliary tract. They may be stained while in the duodenum by the precipitation of bile pigment by the free hydrochloric acid from the stomach.
5. Colon bacilli are occasionally present in the duodenum. The level in the duodenum at which they appear varies.

#### PERNICIOUS ANEMIA

There have been two prevailing theories regarding the origin of pernicious anemia, since it was distinguished from other forms of anemia by Addison. Paul Ehrlich first set forth the theory of "a megaloblastic degeneration of the blood-forming structures," and W. Hunter gave the theory that the process was hemolytic, the origin to be found in a more or less specific affection of the gastro-intestinal tract.

In support of the hemolytic theory, the following discoveries are to be considered:

1. In 1907 Tallqvist showed that in degenerated parts of bothriocephalus, by an autolytic process a cholesterin ester of oleic acid was formed, and that this caused an anemia similar and often identical with the type known as pernicious anemia.
2. In small, nonulcerated cancer of the stomach there is often associated an anemia of megaloblastic type. From these growths Kullmann and Tallqvist have separated a hemolytic substance similar to that obtained in bothriocephalus.
3. Since pernicious anemia is usually associated with and often preceded by digestive disturbances, such as anorexia, gastric discomfort, achylia and periodic diarrhea, Grawitz believed enterotoxic products were formed from insufficient or faulty denaturation of the albuminates, the diarrhea and large amount of mucus being due to a nucleoprotein, formed from the intestinal epithelium.
4. Korshum and Morgenroth produced hemolytic lipoids from normal organs.
5. Berger and Tsuchuja, in two cases reported by Adolf Schmidt, extracted the lipoids from the mucosa of the whole intestinal tract. This they found to have ten times the hemolytic power of that obtained from the normal mucosa.



6. Pilcher, from a study of thirty-four cases of pernicious anemia in the Mayo Clinic, concludes that the achlorhydria resulted from chronic gastritis; that, due to the achlorhydria, the bacterial flora was markedly increased, from which toxins are formed. The absorption of toxins, together with the disturbance of digestion, impairs the formation of antibodies, until finally a bacteremia is produced, usually of streptococcus origin. This stage is recognized by the elevation of temperature and proved by frequently growing the streptococcus from the blood during the febrile period.

7. Experiments have shown that the blood in the peripheral circulation seldom shows signs of hemolysis, or increased fragility. In the portal venous system the excessive accumulation of iron in the liver and the reddish color seen in the portal lymph nodes are suggestive of a hemolytic agent in the portal circulation.

Considering the theory that pernicious anemia is caused by a hemolytic agent and that this agent is dependent on a disturbance of the gastro-intestinal tract, I attempted to find out if there were present in the gastric and duodenal secretions any substance which had a greater hemolytic action than in normal secretions. The hemolytic power of the secretions on the proximal and distal sides of the pylorus was determined on six patients who were not anemic. Another series of six tests on six patients suffering with pernicious anemia was done.

Technic: Into a series of test tubes was placed 5 c.c. of normal saline. Into the first tube 5 c.c. of filtered, fasting gastric or duodenal secretions was added and the contents mixed. Then 5 c.c. of the mixture in the first tube was added to the second tube, which in turn was mixed and the process repeated. This made dilutions of 1 to 1, 1 to 2, 1 to 4, 1 to 8, etc. To each tube 1 c.c. of oxalated, washed red blood corpuscles was added. A control of normal saline and the blood was always made to make sure no hemolysis took place and the presence of oleic acid ruled out. The amount of hemolysis was noted at intervals during twenty-four hours.

The result of this series showed, without a single exception, that the gastric and duodenal secretions from cases of pernicious anemia caused no more hemolysis than do normal secretions.

#### CONCLUSIONS

1. The secretory and motor function of the stomach can best be determined by the fractional method of examination.

2. The normal alkaline duodenal secretion is of a clear, amber color. The presence of free hydrochloric acid precipitates the bile pigments and it becomes of a cloudy yellow color, which will become clear again when neutralized.

3. A cloudy duodenal secretion that is alkaline is significant of an inflammatory condition in the gallbladder, bile ducts, duodenum or stomach. The cloud is due to bacteria and pus. It does not clear on the addition of sodium hydroxid.

4. A detritus that is typical in appearance may be found in the duodenal secretions in cases of cholelithiasis, when there are numerous small stones.

5. An absence of bile pigment and urobilin in the stool is not proof that there is no bile entering the duodenum.

6. Three ounces of bile may be given every day into the duodenum and the stool still give a negative test for bile and urobilin.

7. The secretions on the proximal and distal sides of the pylorus from cases of primary anemia cause no greater hemolysis of red blood corpuscles than the secretions obtained from patients who are not anemic.

# THE RELATION OF HYPERTROPHIC OSTEO- ARTHROPATHY TO PULMONARY TUBERCULOSIS \*

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Cases of hypertrophic pulmonary osteo-arthritis have been presented to this section by Dr. Theodore Janeway and our present secretary, Dr. Brooks. My excuse for bringing the subject to your attention is to emphasize certain points in the relation of this interesting clinical condition to pulmonary tuberculosis.

The relative rarity of osteo-arthritis in association with pulmonary tuberculosis has been mentioned by several authors. The writer of one of our modern textbooks on tuberculosis states that he has examined 2,300 patients suffering from pulmonary tuberculosis without once having encountered an osteo-arthritis. It is only within the past decade that the importance of pulmonary tuberculosis as an etiologic factor in hypertrophic osteo-arthritis has been recognized. The literature to date contains about forty-three cases. Considerable discussion has appeared from time to time regarding the relation of club fingers to hypertrophic osteo-arthritis. A number of French and a few German authors believe that the one bears no relation to the other; but frequently this decision has been reached on insufficient evidence. Bamberger,<sup>1</sup> Marie,<sup>2</sup> and Lefebvre<sup>3</sup> believed both conditions to be similar phenomena. Janeway<sup>4</sup> regarded them as different stages of the same process, and suggested thirteen years ago that careful radiographic observation of the distal ends of the radius and ulna might show slight thickenings in many cases of simple hipocratic fingers.

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\* This work was carried out in the wards of the Montefiore Hospital for Chronic Diseases.

1. Bamberger, E.: Ueber Knochenveränderungen bei chronischen Lungen und Herz Krankheiten, *Ztschr. f. klin. Med.*, 1891, **18**, 193.

2. Marie, P.: De l'osteoarthropathie hypertrophique pneumique, *Rev. de méd.*, 1890, **10**, 50.

3. Lefebvre, A.: Des déformations ostéo-articulaires consécutives à des maladies de l'appareil pleuro-pulmonaire, Thèse de Paris, 1891.

4. Janeway, T.: Hypertrophic Osteo-Arthritis; with Report of Two Cases, *Am. Jour. Med. Sc.*, October, 1903.



In a comprehensive paper published one year ago Dr. Edwin Locke<sup>5</sup> of Boston demonstrated very clearly that simple clubbing of the fingers and hypertrophic osteo-arthropathy were identical, the former representing an early stage of the latter.

It is usually conceded that prolonged venous congestion is the cause of the ordinary clubbed fingers, and Brooks,<sup>6</sup> quoting Ziegler, has pointed out that a continued peripheral hyperemia is produced by compression of the lung capillaries in pulmonary, pleural or mediastinal disease, and that in the course of time this hyperemia induces a



Fig. 1.—Marked increase in the soft tissues of all of the end phalanges. The tips of the distal phalanges present a distinct cauliflower-like formation, and the bases are quite markedly widened, owing to small bony projections. The middle phalanges appear unusually short and their bases are broadened.

hyperplasia in the periosteum and connective tissue. A circulating toxin, the product of the primary disease, probably plays a rôle in producing the bone changes in many cases, and its action appears most apparent where venous stasis has been marked and prolonged.

5. Locke, E.: Secondary Hypertrophic Osteo-Arthropathy and Its Relation to Simple Club-Fingers, *THE ARCHIVES INT. MED.*, 1915, **15**, 659.

6. Brooks, Harlow: The Etiology of Hypertrophic Pulmonary Osteo-Arthropathy, *New York Med. Jour.*, Sept. 27 and Oct. 4, 1913.

The underlying changes in the soft tissues of the fingers and toes have been generally described as a connective tissue hyperplasia, with capillary dilatation. The essential pathologic features of the bone disease consist in a progressive, ossifying periostitis, usually manifesting itself in the distal ends of the diaphyses of the long bones of the arms and legs, and later involving the other bones of the skeleton. In long-standing cases the changes in the periosteum may involve all of the bones, including the cranial bones. These bone changes in advanced



Fig. 2.—The soft tissues of all the toes are increased and the tips of the distal phalanges of the great toes show an exaggeration of a burr-like formation. The fifth metatarsal bones appear at their inner aspect to be lined with a thin layer of new bone, perfectly regular in outline, and showing a sharp line of demarcation between the new bone and cortex.

stages of the disease are not necessarily confined to the diaphysis, but, as Locke has shown, the epiphyseal portions may produce new bone formation. We were unable to demonstrate this in any of our roentgenograms, but none of our patients presented so pronounced a picture of the disease as some of those described by Locke.

Patients presenting evidence of hypertrophic osteo-arthritis vary markedly in their clinical manifestations of the disease. Some

show simple hypertrophy of the soft tissues about the terminal phalanges, with thickening and cyanosis of the nails, which are at times parrot beak in shape; others, in addition to this clubbing, display an enlargement of the hands and feet, and a thickened, clumsy appearance of the lower forearms and lower legs. When the disease is well developed, the normal tapering appearance of the lower forearms and lower legs is completely lost, the periarticular thickening is marked, and the joint involvement pronounced. The patients sometimes complain of



Fig. 3.—Apparent increase of soft tissues about the terminal phalanges. The tips of these phalanges show a burr-like formation. The fifth metatarsal bones present a distinct layer of new bone, about 0.2 cm. in width, on the internal aspect.

considerable pain in the bones and joints involved and examination often shows these parts to be quite tender.

A point mentioned by the earlier writers and well emphasized by Brooks is the tendency in these patients to the formation of a globular nose and distinct evidence of thickening of the subcutaneous tissue in the malar regions. These marks of peripheral stasis occurred quite prominently in our series of patients, the changes in the nasal tip being present in twelve patients, and the malar thickening appearing in nine of them.



The thirty-two patients to be reported on were gathered from a service of about 100 patients suffering from pulmonary tuberculosis. They were all in the second or third stage of the disease. Roentgen examinations were made of the hands, feet, all of the long bones, the bones of the pelvis, and in some instances the shoulder girdle and cranial bones. Following the classification made by Locke, we have divided these thirty-two patients into three groups (Tables 1, 2 and 3):



Fig. 4.—The fifth metatarsal bones show on their inner aspect a thin layer of new bone about 0.2 cm. at their broadest point. A sharp line of demarcation separates the new bone from the cortex of the metatarsal.

(1) five patients presenting simple, well-defined clubbing of the fingers without bone changes; (2) seventeen patients showing clubbing of the fingers with bone changes in the phalanges; and (3) ten patients with clubbed fingers and changes in the long bones.

Of the five patients in Group 1, namely, those presenting simple clubbing of the fingers, three were men and two women. They varied in age from 19 to 42 years, and the length of illness ranged from one

to six years. Extensive pulmonary cavitation was present in three of these patients (M. V., S. L. and J. M.). It is noteworthy that the patient J. M., who had been ill six years, first noticed a change in the appearance of her fingers three and a half years ago. Roentgen-ray examination, made only one month before her death, failed to demonstrate the slightest change in any of her bones, in spite of the presence of an extensive pulmonary lesion, which, during the last year of her life, had produced dyspnea, toxemia, and long periods of continued fever. The patient M. V. presented a similar history, extending over a shorter period, with the same negative Roentgen-ray findings.



Fig. 5.—Section of lower end of radius from patient I. G., showing a dense layer of new bone formation separating the periosteum from the cortex.

Of the seventeen patients in Group 2, fourteen were men and three women. The ages varied from 18 to 63 years, and the length of illness from one to six years.

The appearance of the patients in this group differed in no way from those in Group 1. They presented various degrees of clubbing of the terminal phalanges of the fingers and toes, but the Roentgen-ray examination disclosed changes in the bones of the hands and feet not seen in those of Group 1. The bone changes consisted in cauli-flower-like excrescences of the tips of the end phalanges, with point-like projections of the bases of those phalanges. At times these projections appeared as true osteophytes. The middle phalanges were short, thick and broadened at the base (Fig. 1).

The Roentgen-ray examination in three of these patients showed a layer of new bone formation along the lateral or internal aspects of the fifth metatarsal bones (Figs. 2, 3 and 4). In the patient D. H. section of the fifth metatarsal proved the inner layers of the periosteum to have been invaded by a new bone formation, the new tissue differing from the old bone in that the osteoblasts were few in number and showed no lamination (Fig. 5).



Fig. 6.—The fifth left metatarsal bone presents at its lateral aspects a thin layer of new bone, showing in some places a distinct line of demarcation. This line disappears entirely at the middle portions, where the normal differentiation between the cortex and medullary layer has disappeared.

All of the patients in Group 3 were men. The age range was between 25 and 43 years, and the length of illness from less than one to eleven years.

In three of these patients the lower ends of the forearms and lower portions of the legs were markedly thickened and clumsy in appearance. In two of these patients the thickened areas were distinctly tender. Although one patient complained of pains in his wrists and ankles, there was no apparent change in the joints of any.



Six patients of the ten in this group presented the globular nose formation and malar thickening. With the exception of the awkward appearance of the lower arms and legs in the three patients just described, the patients in Group 3 disclosed nothing in their physical examination to differentiate them from patients in either of the other two groups, yet the Roentgen-ray examination revealed decided differences. Nine of them showed changes in the phalanges similar to those described as characteristic of Group 2. A layer of new bone

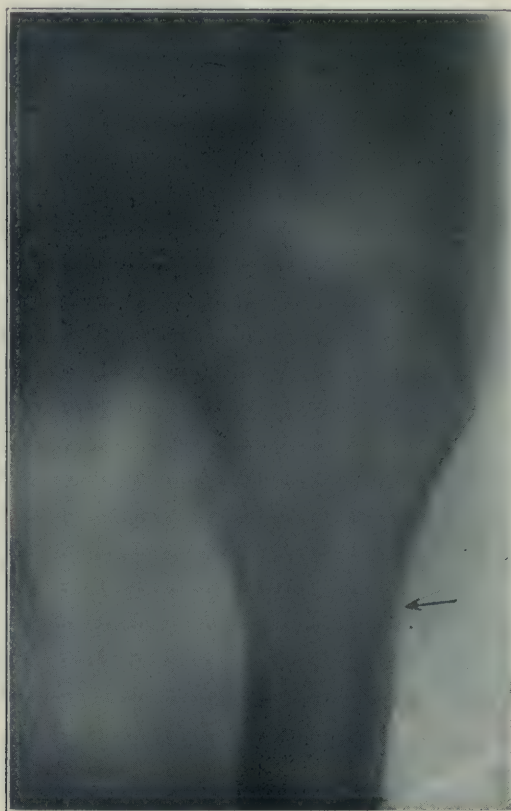


Fig. 7.—The upper third of the femur shows a thick layer of dense and well-differentiated new bone at the lateral and external aspect.

formation on either the inner or outer aspect of the fifth metacarpal or metatarsal bone was evident in six patients of this group (Fig. 6). We were unable to determine an anatomic basis for the appearance of this new subperiosteal bone formation in the fifth and not in any of the other metacarpal or metatarsal bones.

All of the patients in this group showed more or less marked changes in the bones of the forearms and legs, and in three patients the

femora were involved (Fig. 7). The subperiosteal layer of new bone, usually about 0.5 cm. in thickness, appeared at the epiphyseal line or just above and extended upward for varying distances (from 4 to 10 cm.), in most instances showing a sharp line of demarcation from the cortex (Figs. 8, 9 and 10). In other patients the layer of new bone merged at points with the old, and in some a lack of differentiation between the cortical and medullary layer of the bone proper was



Fig. 8.—Layer of new bone just above the styloid process of the ulna and extending upward about 4 cm.

evident (Fig. 11). Plates taken in several positions demonstrated that this layer of subperiosteal new bone appeared on the various aspects of the bone involved.

One of these patients (A. G.) showed marked clubbing of his fingers without changes in the distal phalanges, but a distinct layer of subperiosteal new bone was demonstrated in the ulna, tibia and fibula. Such findings emphasize the importance of Roentgen-ray examinations

of the long bones, even in those cases which fail to reveal pathologic conditions in the phalanges; and they are also of aid in correcting many of the earlier misconceptions regarding the pathogenesis of this condition.

We sought in this whole series of patients to establish some relation between the clinical manifestations of the pulmonary disease and the type of osteo-arthropathy present, but we have been unable to demonstrate a connection between the two.



Fig. 9.—Both femora present symmetrical changes consisting of a layer of new bone at both lateral aspects, beginning just above the condyles and extending upward on the external side about 5 cm. and on the internal side about 3 cm.

A study of the tables will show that pronounced evidence of pulmonary tuberculosis may be associated, on the one hand, with simple clubbing of the fingers, while, on the other hand, less marked manifestations of the pulmonary disease may be accompanied by distinct changes in the long bones. The bone changes, moreover, are not definitely associated with the type or degree of the clinical expression of the pulmonary disease.



Very few reports of metabolic studies of patients suffering from hypertrophic osteo-arthropathy have appeared in the literature. Through the courtesy of Dr. Brooks we were enabled to investigate the metabolism in the patient exhibited by him before this section in 1911. This patient presented a very advanced type of the disease, so that we thought if a perversion of metabolism existed in osteo-arthropathy, it could easily be demonstrated in this patient. A careful



Fig. 10.—The ulna shows at its external aspect a fine layer of new bone formation, extending from the styloid process upward about 3 cm. Practically the same change is seen at the external aspect of the radius.

study of his nitrogen and calcium metabolism, carried out for one week by Dr. Janney, failed to show a deviation from normal standards.

When we studied these three groups of patients in connection with the Roentgen-ray examinations, certain facts became evident:

1. The degree of clubbing corresponds only in a general way to the radiographic findings.

2. Bone changes occur more commonly than is ordinarily supposed in the so-called simple-clubbed fingers.

3. Pronounced changes in the long bones may take place without any clinical sign or symptom being present.

4. Systematic Roentgen-ray examinations reveal such changes.



Fig. 11.—Dense layer of new bone lining both lateral aspects and extending upward above the malleoli about 6 cm. The normal line of differentiation between the cortical and medullary bone has to a great extent disappeared at the lower end of the tibia and fibula.

5. The type of bone change in the various groups is the same, the difference being rather in the degree.

6. Hypertrophic osteo-arthropathy is present more frequently in pulmonary tuberculosis than the usual clinical examination would permit us to believe.

## GROUP 1.—FIVE PATIENTS PRESENTING SIMPLE, WELL-DEFINED CLUBBING OF THE FINGERS WITHOUT BONE CHANGES

CASE 1 (No. 27922).—H. L., aged 32 years, a Russian, presented a lung condition dating back two years and beginning with a sudden hemoptysis. He was in the third stage of an afebrile course, with the sputum positive.

The index and middle fingers of the left hand showed a fair amount of clubbing with curved nails. He did not complain of any joint pains, nor was he aware of his digital abnormalities.

The Roentgen-ray report on the bones showed that there is a distinct increase in the soft parts of all the fingers, most marked at the tips and around the interphalangeal joints. There was also marked increase in the soft parts of all the toes, but there were no bone changes in hands, feet, arms or legs.

CASE 2.—M. V., a man, aged 42 years, an Austrian, a cutter by occupation, was examined Nov. 23, 1915, and presented a lung condition which dated back two years. He was in the third stage with the sputum positive, and the disease was running a febrile course. Both upper lobes were infiltrated and moist râles were present. The disease was complicated by the loss of the superficial epithelium of the right false vocal cords and perforation of the lower quadrant of the left ear drum. The patient had never noted any changes in the distal ends of the extremities until his attention was called to it. The heart was slightly dropped.

TABLE 1 (GROUP 1).—FIVE PATIENTS PRESENTING SIMPLE CLUBBING OF FINGERS AND TOES

Name	Age, Yr.	Years Ill	Stage	Fever	Dyspnea	Toxemia	Cough	Expectoration
H. L.	32	2	3	0	Marked	Moderate	Marked	Marked
M. V.	42	2	3	+	Marked	Marked	Severe	Profuse
S. L.	23	6	3	+	Marked	Slight	Severe	Profuse
D. R.	19	1	2	Slight	None	Slight	Moderate	Moderate
J. M.	23	6	3	+	Marked	Marked	Marked	Profuse

The finger nails were moderately curved and showed longitudinal striations and some thickening. There was slight enlargement of the distal phalanges as a whole. These changes were still more marked in the feet, more particularly in the great toes, in which the thickening of the soft tissue was very evident.

The Roentgen-ray report did not show any pathologic changes in the terminal phalanges or other bones of the hands, feet, arms or legs.

CASE 3.—S. L., a Russian woman, aged 23, a milliner by occupation, presented a lung condition which dated back six years. Both upper lobes were infiltrated with evidences of activity throughout both lungs. The patient was in the third stage of the disease, which was running a febrile course. The sputum was positive.

The patient had had no pain in any of the joints of the extremities. She did not remember having noted any change in the distal ends of the extremities. The nails of the fingers were thickened, somewhat parrot beak in shape, and the fingers and toes were slightly clubbed.

The Roentgen-ray report showed no pathologic changes in the bones of the hands, feet, arms or legs.

CASE 4.—D. H., a newsboy, aged 19 years, born in the United States, showed a pulmonary condition which dated back one year. He also suffered from a cardiac disturbance necessitating his stay in bed. His pulmonary disease was



in the third stage, and the sputum was positive. The disease was running an afebrile course.

There was slight curving of the nails and very slight thickening of the soft tissue, with similar changes, but more marked, in the toes, particularly in the great toes.

The Roentgen-ray report showed that there was apparent increase in the soft tissue of the hands, but no bone changes in the hands or feet. The forearms, arms, legs and femora were negative.

CASE 5.—J. M., a woman, aged 23, showed a pulmonary condition which dated back six years. She was in the third stage, running a febrile course, with the sputum positive.

The disease was complicated by tuberculous enteritis, laryngitis and nephritis.

The patient first noticed changes in the shape of her fingers and toes about three and a half years before examination. She had had no pain in any joints until a short time before examination. The pains she complains of were only of the toes, ankles and feet, probably due to the pressure exerted by the edema present. There was slight curving of the nails and clubbing of both the fingers and the toes.

The Roentgen-ray report showed that the marked increase was in the soft tissues of the fingers and toes, but that there were no bone changes in the hands, feet, arms or legs.

#### GROUP 2.—SEVENTEEN PATIENTS SHOWING CLUBBING OF THE FINGERS, WITH BONE CHANGES IN THE PHALANGES

CASE 1 (No. 25610).—S. K., a man, aged 55 years, a pedler by occupation, was examined Nov. 23, 1915, and gave a pulmonary history dating back four years. He was in the second stage of an active process, usually afebrile. There was emphysema, and the sputum was positive.

He had had no pain in any of the joints, but he had noticed a slight clubbing of the fingers and curving of the nails about twelve years before, which gradually became more marked as the pulmonary disease progressed.

There was a slight clubbing of the fingers and curving and thickening of the finger nails, particularly of those on the right hand, also a slight enlargement of all toe tips and curving of the toe nails. There was apparent enlargement of the nasal tip, which was somewhat globular in shape.

The Roentgen-ray report showed cauliflower-like growth of the tips of the terminal phalanges, and Heberden's nodes were present, but there were no other pathologic changes in the bones of the extremities.

CASE 2 (No. 29158).—J. M., a man, aged 27, a salesman by occupation, was examined Aug. 19, 1915, and showed a pulmonary condition which dated back about three years. He was in the second stage, with both upper lobes of the lungs infiltrated, and the sputum positive.

He had not had pain in any joint, and did not remember when the joint enlargement began. The only enlargement of joints was found in the distal ends of the phalanges. These were slightly clubbed, with thickening of nails, which were also slightly curved.

The Roentgen-ray report showed marked cauliflower formation at the tips of all the end phalanges of the hands. Distinct bony excrescences were visible at the bases of the middle and end phalanges of the second, third, fourth and fifth fingers on both sides, giving those basal portions a broadened, somewhat fan-shaped appearance. There were a few minute arthritic changes at some of the distal interphalangeal joints. The feet, legs and forearms gave no definite evidence of any pathologic changes in any of the bones.

CASE 3 (No. 31071).—J. L., a tailor, aged 51, showed a pulmonary condition which dated back one year. He had had no pains in the joints or the long bones. He had at the time of examination pains in both calves, but he had had no rheumatism or sore throat. There were distinct curving and thicken-

ing of his nails and slight hypertrophy of the soft parts of his finger tips, with marked thickening and curving of the nails of the toes.

Both upper lobes of the lungs were dull, with no apparent excavation. He was running an occasionally febrile course, and he had had pulmonary hemorrhages at times. He was in the second stage, with sputum positive. The heart was small, somewhat dropped, and there was moderate bulging of the right wall of the ascending aorta.

The Roentgen-ray report showed that the tips of the end phalanges of the hands had an exaggeration of burr-like excrescences, and their bases had point-like projections which might be called abnormal. There was a moderate amount of cystic degeneration at the tips of the end phalanges. The forearms, arms and lower extremities were negative.

TABLE 2 (GROUP 2).—SEVENTEEN PATIENTS PRESENTING SIMPLE CLUBBING OF FINGERS OR TOES WITH CHANGES IN THE DISTAL PHALANGES

Name	Age, Yr.	Years Ill	Stage	Fever	Dyspnea	Toxemia	Cough	Expectoration
S. K.	55	4	2	At times	Moderate	Slight	Marked	Profuse
J. M.	27	3	2	At times	Moderate	Slight	Moderate	Moderate
J. L.	51	1	2	Very slight	0	0	Moderate	Moderate
M. E.	42	3	3	+	+	+	Severe	Profuse
J. F.	63	4	3	Slight at times	+	+	Severe	Profuse
J. J.	29	2	3	Slight	0	Slight	Moderate	Moderate
E. B.	34	4	3	Slight at times	0	0	Moderate	Profuse
T. R.	40	2	3	At times	Slight	Slight	Severe	Profuse
M. B.	18	1½	3	+	+	+	Severe	Moderate
J. S.	50	4	3	At times	0	0	Slight	Very moderate
S. J.	37	1	3	+	+	+	Severe	Profuse
D. H.	45	2	3	+	+	+	Severe	Profuse
N. S.	45	5	3	+	Slight	Moderate	Severe	Profuse
M. C.	42	3½	3	0	Slight	Moderate	Severe	Profuse
I. S.	30	2½	3	At times	0	Slight	Severe	Profuse
A. S.	39	3	3	+	+	+	Severe	Profuse
P. M.	23	6	3	Slight	Slight	Slight	Moderate	Moderate

CASE 4.—M. E., a Russian, aged 42 years, a hatter by occupation, showed a lung condition which dated back three years. He was in the third stage of pulmonary tuberculosis, which was complicated by laryngitis and hemoptysis. The sputum was positive, the course of the disease febrile.

He had noted no change in the appearance of his finger tips, nor had he experienced any joint pains. The nasal tip was thickened, and there was well-marked clubbing of his thumbs. This was less evident in the other fingers.

The Roentgen-ray examination showed that not only were the soft parts of all the fingers greatly increased, but the bony parts showed very marked cauliflower excrescences at the tips of the end phalanges and marked point-like projections of the basal portions of the same phalanges. The middle phalanges appeared short, thick, and markedly broadened at the basal portions. The forearms, feet and legs were negative.

CASE 5.—J. F., a Russian, aged 63, a ragpicker by occupation, was examined Oct. 1, 1915, and showed a lung condition which dated back four years. He



was in the third stage, with the sputum positive, and the disease running an afebrile course.

Attention was first drawn to the clubbed condition of the finger tips about two years before, and this had afterward increased. He had no pains in the fingers. Both hands showed the same condition. The joints had never been painful. Examination revealed well-marked clubbing and incurving of the nails of all the fingers and of the second right toe and the first and second toes of the left foot. The patient complained of pains over lowest portion of the sternum on pressure. There was also marked thickening of the nasal tip.

Roentgen-ray examination showed a moderate amount of burring at the tips of the terminal phalanges. Point-like osteophytes were seen at the bases of most of the middle and end phalanges. There were beginning arthritic changes at some of the middle and distal interphalangeal joints, most marked in the fifth fingers. The feet, leg and forearms showed no definite bone changes. There was a beginning arteriosclerosis.

CASE 6.—J. J., a Russian, aged 29, a woodcarver by occupation, showed a condition which dated back two years. He was in the third stage, the sputum was positive, the course febrile. The finger tips were moderately clubbed and the nails were curved; the toes were negative.

By Roentgen-ray examination the tips of the end phalanges of almost all the fingers showed a moderate amount of cauliflower formation. The basal portions of the end and the middle phalanges showed a point-like excrescence. The forearms, arms, feet and legs were negative.

CASE 7.—E. B., a Russian, aged 34, a musician by occupation, gave a pulmonary history which dated back to March, 1912, when he began to expectorate blood-streaked sputum. He was in the second stage, the sputum was positive, and the course of the disease was afebrile. The finger tips show a fairly well-marked clubbing and incurving of the nails of both hands. The patient was not aware of this until his attention was called to it. The fingers, as a whole, looked rather short and thick. The nose was globular, and there was a thickening of the subcutaneous tissue in the malar region. The toes were similarly affected, but to a lesser degree. At no time had there been pain in the joints or extremities.

The Roentgen-ray report, made Sept. 8, 1915, showed a moderate amount of burring at the tips of all the end phalanges of the hands. There was a slight tendency to Heberden's node formation. The feet showed a moderate, irregular thickening of the cortical layer at the outer aspect of the upper third of the second left metatarsus, about one inch in extent. The legs and forearms gave no evidence of any gross bony lesion.

CASE 8 (No. 31309).—T. R., a Russian, aged 40, suffered with an illness which dated back two years. He was in the third stage of pulmonary tuberculosis, running a febrile course, and the sputum was positive. There was a tendency to globular nose, but no malar thickening. The nails were curved, slightly cyanosed and somewhat thickened. There was marked thickening of the soft tissue of the terminal phalanges of the hands. The changes in the feet were similar, but less marked than in the fingers.

The Roentgen-ray examination showed that there was marked increase of all the soft parts in the fingers, most marked at the end phalanges. The bony parts showed a distinct broadening at the basal portion of the middle and distal phalanges. Most of the interphalangeal joints showed a distinct narrowing of the articular clefts. There were no bone changes in the arms, feet or legs.

CASE 9 (No. 30389).—M. B., a Russian, aged 18 years, was examined Aug. 30, 1915. The patient's lung condition dated back one and one-half years. He was in the third stage of pulmonary tuberculosis, with positive sputum, and running a febrile course. There was an increase in the soft tissue of the fingers and a distinct thickening of the soft parts of the toes.



The Roentgen-ray examination showed that there was marked increase in the soft tissues of all the end phalanges of the hand, with the beginning of cauliflower formation and cystic degeneration in the bony tips. The forearms, arms, feet and legs were negative.

CASE 10 (No. 30407).—N. S., a man, aged 45 years, a cleaner by occupation, presented a pulmonary condition which dated back five years. At no time had he had pain in any of the joints, and he did not remember when the joint enlargement began. He was in the third stage of pulmonary tuberculosis, complicated by laryngitis, running a febrile course. The sputum was positive. There was marked thickening of the nails with longitudinal striations and parrot beak curving and hypertrophy of the soft tissues.

The Roentgen-ray examination, made Sept. 24, 1915, showed the hands with very marked cauliflower formation at the tips of all the phalanges. The basal portions of most of the terminal and some of the middle phalanges showed slight osteophyte formation. Most of the middle and terminal phalanges presented a peculiar fan-shaped appearance. The feet, legs and forearms showed no definite bone changes.

CASE 11 (No. 28386).—M. C., a Russian, aged 42 years, a painter by occupation, showed a pulmonary condition which dated back three and one-half years. He was in the third stage of pulmonary tuberculosis, running an afebrile course. The sputum was positive. There was slight thickening of the nasal tip and the subcutaneous tissue of the malar region. There were also distinct thickening and curving and longitudinal striations of the finger nails, and moderate thickening of the soft tissue, with similar changes in the toes.

The Roentgen-ray examination showed that there was a marked increase of the soft tissues in all the fingers, most marked in the tips, which also had an exaggerated cauliflower appearance. The bases were broadened, due to lateral excrescences, and the middle phalanges appeared somewhat shortened and thickened, due to an enlargement of the basal portions. The forearms and arms were negative. The soft tissues of the big toe were distinctly increased and the tips of the end phalanges showed marked burr-like excrescences. The legs were negative.

CASE 12.—A. S., an Austrian woman, aged 29 years, houseworker, showed a condition which dated back three years. She was in the third stage of pulmonary tuberculosis, running a febrile course. The sputum was positive. The patient had not noted a change in any of her fingers or toes, nor did she ever complain of any pain in the bones or joints. There was slight curving of the nails and clubbing of the finger tips, but there were no similar changes in the toes.

Roentgen-ray examination showed that the soft parts of all the fingers were markedly increased, especially at the end phalanges and around the interphalangeal joints. The bony parts presented very marked cauliflower formation at the tips of the end phalanges, marked broadening of the nasal portions of the same phalanges, due to bone excrescences. The basal portions of all the middle phalanges showed marked increase in width. All the other bones of the lower extremities failed to show any abnormality.

CASE 13 (No. 32783).—P. M., aged 23 years, a clerk, born in the United States, showed a pulmonary condition which dated back six years. He was in the third stage of pulmonary tuberculosis. The nasal tip showed some thickening. There was distinct curving and thickening with cyanosis of the finger nails, and moderate clubbing of the finger tips. There was very slight curving of the toe nails, with cyanosis; some thickening of the soft tissues of the tips, but they were not distinctly clubbed. There was no thickening or deformity of the long bones.

Roentgen-ray examination showed a moderate amount of bony overgrowth at the tips of the distal phalanges, with slight point-like projections at the bases of the same phalanges. There were no bone changes in the feet, arms or legs.

CASE 14.—I. S., a housewife, aged 30 years, an Austrian by birth, gave a pulmonary history which dated back two and one-half years. She was in the third stage of pulmonary tuberculosis, which was running a febrile course. The sputum was positive. There was no history of pain in any of the joints. There was slight clubbing of the fingers.

Under Roentgen ray the basal portions of most of the phalanges of the hands showed fine point-like projections. The fifth metatarsal bones showed a distinct irregularity in outline along both lateral aspects, extending almost throughout the entire shaft. This condition is symmetrical. The forearms, arms, legs, femora, hips and pelvis were negative.

CASE 15 (No. 31464).—D. H., aged 45 years, a tinsmith by occupation, gave a pulmonary history dating back to July, 1914. He was in the third stage of pulmonary tuberculosis, complicated by acute tuberculous pneumonia, tuberculous ulcer of the tongue, and tuberculous laryngitis. The sputum was positive. There was marked thickening and curving of the finger nails and a moderate hypertrophy of the soft tissues of the fingers. There were similar but less marked changes in the toes.

Roentgen-ray examination showed that the soft parts of all the fingers were moderately increased and the tips of the end phalanges showed quite a marked cauliflower appearance. The fifth metacarpal bones presented marked irregularity at the external and lateral aspects of the shaft, due apparently to irregularly formed layers of new bone. The forearms, feet and legs were negative.

The pathological report gave an anatomic diagnosis of calcified mediastinal lymph gland, chronic ulcerative pulmonary tuberculosis, proliferative miliary tubercles (lung), chronic and acute tuberculous pleuritis, acute tuberculous pneumonia, tuberculous laryngitis, tuberculosis of epididymis and seminal vesicle, tuberculous ulcer of tongue, tuberculous ulcer of colon, anemia and emaciation, fat deposits in the liver and pulmonary edema.

Microscopically a metatarsal section showed the inner layers of the periosteum invaded by new bone. This tissue differed from the old bone in that the matrix stained blue, the osteoblasts were few in number and showed no lamination.

The pathologic work in connection with this study was performed by Dr. Klein in the pathologic department of the Montefiore Hospital for chronic diseases.

CASE 16 (No. 30482).—S. J., aged 27 years, a tailor by occupation, gave a pulmonary history dating back one year. He had had no pain in any of the joints nor had he noticed any change in appearance of his fingers or toes. He was in the third stage of pulmonary tuberculosis and was running a febrile course. The sputum was positive. There was moderate thickening and curving of the finger nails and slight cyanosis. All the nails showed longitudinal striations and the finger tips showed thickening. Similar changes were evident in the toes. There was no enlargement or tenderness of the lower ends of the arms or legs.

Roentgen-ray examination, made Sept. 27, 1915, revealed a moderate amount of cauliflower formation at the tips of the terminal phalanges. There was some tendency to Heberden's node formation and some broadening of most of the middle and end phalanges. The feet were negative. There was marked irregularity in outline of the lower end of the right radius, and separation of the styloid process of the right ulna, due apparently to an old Colles' fracture of this process. The legs gave no evidence of any gross bony lesion.

CASE 17 (No. 29600).—J. S., a man, aged 50 years, a furrier by occupation, gave a pulmonary history dating back four years. He was in the third stage of pulmonary tuberculosis, running an occasionally febrile course. The sputum was positive.

For the previous two years the patient had occasional pains in his shoulder joints. These became constant during the previous year. Five years before



he had some throat trouble, with a constant burning pain. This had disappeared about two years before examination. He had never noticed a changed appearance of his fingers or toes. There was a thickening of the tip of the nose and of the subcutaneous tissue of the malar region, also clubbing of the fingers as well as slight enlargement of all the toes.

Under Roentgen ray the tips of all the end phalanges of the hands showed an amount of cauliflower formation, and the basal portions were broadened, due to point-like bony excrescences.

The fifth metacarpal bones, especially on the right side, showed marked irregularity and thickening of the cortical portions of the shaft at the lateral and external aspect. The forearms, feet and legs showed no definite abnormalities.

#### GROUP 3.—TEN PATIENTS WITH CLUBBED FINGERS AND CHANGES IN THE LONG BONES

CASE 1 (No. 29294).—S. S., aged 43, a peddler by occupation, gave a pulmonary history dating back four years. He had never had pain in any of the joints, but he had had pains in the soles of his feet. He was in the third stage of pulmonary tuberculosis, which was running a usually afebrile course. The sputum was positive. There was distinct thickening and slight cyanosis of the finger nails. The thumb nails were markedly curved and showed deep longitudinal striations. The tips of distal phalanges showed a cauliflower-like formation. The bases of the terminal and second phalanges were broadened. The forearms were negative. The feet failed to show any definite change. Both tibiae showed a dense layer of new periosteal bone extending from the malleolus region upward along the lateral aspect of the shaft about 4 cm., appearing quite well differentiated from the bone proper almost throughout the entire extent.

CASE 2 (No. 29997).—L. C., aged 39, a tailor by occupation, showed a pulmonary condition which dated back about eleven years. He was in the third stage of pulmonary tuberculosis, which was running a usually afebrile course. The sputum was positive. The disease was complicated by perforation of drum of left ear, nephritis and right inguinal hernia. The patient had never noticed any changes in the shape of the distal ends of his fingers or toes. He had complained of pains in his wrists, carpal, metacarpal, and phalangeal joints, and in the ankle, tarsal, and metatarsal joints during the previous year. He had never had rheumatism. The tip of his nose was thick and bulbous, and the integument in the malar region was thickened. There were typical parrot-beak nails, and fingers and toes were typically clubbed. There was distinct thickening of the legs below the calves, so that the ankle looked heavy and clumsy. This was more marked on the right side. This region was not tender.

Roentgen ray showed quite marked increase in the soft parts of all the fingers, most noticeable at the tips. The bony parts showed a marked cauliflower appearance at the tips of the end phalanges, with marked broadening of the basal portions, due to point-like projections. The forearms and arms were negative. The fifth metatarsal bones showed a thin layer of periosteal new bone, lining the shaft at the internal aspect and being quite well differentiated from the bone proper. Both fibulae and tibiae showed a very dense and quite thick layer of new bone lining both lateral aspects and extending upward above the malleoli about 6 cm. The lower thirds of the tibiae showed a marked lack of differentiation between cortical and medullary layer of bone. This layer of new bone formation was at its widest part about 0.5 cm. in thickness, and in some places not differentiated from the bone proper, which gave the lower ends of fibulae and tibiae a clumsy and misshapened appearance. The lower half of the femora showed at the anterior aspects a fine but distinctly differentiated layer of periosteal new bone. The upper thirds of both femora show quite a thick layer of dense and well-differentiated new bone



at the lateral and external aspect. This layer was about  $\frac{1}{8}$  cm. at its widest place. The joints were free and the hips and iliac crests were negative.

CASE 3 (No. 31603).—A. G., aged 25, a musician by occupation, presented a pulmonary condition dating back four years. He was in the third stage of pulmonary tuberculosis, usually afebrile. The sputum was positive. He had never had pain in any of the joints, and had never noticed the clubbing of the fingers and curving of the nails. There was present clubbing of the fingers and toes, with curving of the nails of both.

The Roentgen-ray examination revealed marked increase in the soft tissues of the end phalanges of the hands, with a point-like projection at the bases. Most of the middle and distal phalanges showed marked broadening of the bases. Most of the hand bones showed advanced irregular atrophy. The left ulna showed at its lower end, extending just above the styloid process upward about 3 cm., a thin layer of new bone formation with a sharp line of demarcation. The upper arms and shoulders were negative. The fifth metatarsal bones were lined at the internal aspect with a distinct layer of new bone for-

TABLE 3 (GROUP 3).—TEN PATIENTS WITH CLUBBED FINGERS AND CHANGES IN THE LONG BONES

Name	Age, Yr.	Years Ill	Stage	Fever	Dyspnea	Toxemia	Cough	Expectoration
L. C.	39	11	3	0	0	0	Moderate	Moderate
S. S.	43	4	3	Slight at times	0	0	Moderate	Moderate
I. G.	38	4	3	Slight	Slight	+	Severe	Profuse
A. G.	25	4	3	Slight at times	0	Slight	Moderate	Moderate
L. R.	38	3	3	At times	At times	Slight	Severe	Profuse
H. S.	38	7	3	0	0	0	Moderate	Moderate
N. C.	35	4*	3	+	+	+	Moderate	Moderate
I. A.	35	5*	3	+	Slight	Moderate	Severe	Profuse
J. M.	36	3*	3	Occasional	Slight on exertion	Moderate	Severe	Profuse
S. A.	32	5*	3	+	Slight	Moderate	Severe	Profuse

\* Period of illness expressed in months.

mation about 0.2 cm. at its widest. There was marked increase in the soft tissues of all the toes. The lower end of tibia and fibula showed at their lateral aspect, beginning just above the malleoli line and extending upward about 4 cm., a more or less fine layer of new bone, merging gradually into the normal cortical layer, showing quite a distinct line of demarcation. The upper third of the fibula is lined with quite a thick layer of periosteal new bone about 0.25 cm. in thickness, in some places showing a demarcation line, in others merging with the cortical layer of the fibula. The femora and pelvic bones were negative.

CASE 4.—H. S., a Roumanian, aged 38 years, presented a lung condition which dated back seven years. He was in the second stage of pulmonary tuberculosis, running an afebrile course. The sputum was positive. There is well-marked clubbing of the fingers and toes.

Roentgen-ray examination showed that the soft tissues of all the end phalanges of the hands were greatly increased. The tips showed a moderate amount of cauliflower formation. The bases and phalanges were markedly broadened, especially in the little fingers, where there are quite marked point-like bony excrescences. The middle phalanges of almost all the fingers appear abnormally short and their bases distinctly widened. The forearms were

negative, except for a slight irregularity at the lateral aspect of the right radius just above the styloid process. The right radius presented at the aspect of its lower end, beginning one-half inch above the styloid process and extending about 2 inches, an irregularly outlined, thin fine layer of new bone. The feet were negative. The legs were negative, except for a moderate amount of irregularity of outlines in the fibula just above the lower tibiofibular joints to the extent of about 2 cm. The knees showed slight arthritic changes at the patella. The femora and pelvic bones were negative.

CASE 5 (No. 31985).—N. C., aged 35 years, a painter by occupation, showed a pulmonary condition dating back four months. He was in the third stage of pulmonary tuberculosis, running a febrile course. Sputum positive. He had never had pain in any of the joints; he had noticed enlargement of the fingers and curving of the nails for about one year. There was marked clubbing of the fingers and curving of nails, also slight enlargement of all the toes and curving of the toe nails. The lower ends of the tibiae are thickened but not tender. The patient died Nov. 11, 1915.

Roentgen-ray examination showed a very marked increase in the soft tissues of all the end phalanges. The tips of the bony end phalanges presented a distinct cauliflower formation and the bases were quite markedly widened, due to a little bony projection. The middle phalanges appeared unusually short and their bases were broadened. The rest of the bones of the hands appeared normal. The ulnae showed at their external aspects a fine layer of periosteal new bone formation extending from the styloid process upward about 3 cm. Practically the same change was seen at the external aspects of the radii. These radial and ulnar changes were symmetrical. The upper arms were negative. In the feet there was an apparent increase of soft tissues in the end phalanges, with burr-like bony outgrowths at the tips. The fifth metatarsal bone shows along its inner aspect a thin layer of periosteal new bone formation about 0.2 cm. at its widest, and presenting a sharp line of demarcation. Roentgenograms taken in various positions show that the lower thirds of tibiae and fibulae were covered with a layer of new bone formation about 0.25 cm. at its widest, and showing everywhere a sharp line of demarcation. Higher up this new-formed bone merges gradually with the normal cortex. The changes in the fibulae extend a little farther upward than in the tibiae. The contiguous portion of the tibia and fibula at the lower tibiofibular joint showed marked irregularity in outlines. The upper thirds of the fibulae, beginning just below the upper tibiofibular joint, showed a distinct thin layer of a newly formed bone at its anterior aspect. Both femora showed symmetrical changes consisting of a layer of periosteal bone at both lateral aspects, beginning just above the condyles and extending upward on the external side about 5 cm. and the internal side about 3 cm. The middle thirds of both femora show an irregularly outlined layer of new bone at both lateral aspects, about 0.5 cm. in thickness. The hips and pelvic bones were negative.

CASE 6 (No. 30529).—I. A., aged 35 years, gave a pulmonary history dating back five years. He was in the third stage of pulmonary tuberculosis, which was running a febrile course. The sputum was positive. He had never had pains in any of the joints, nor had he noticed clubbing of the fingers or curving of the nails. The nails were distinctly parrot beak in shape, thickened, cyanotic, and showed longitudinal striations. The finger tips were somewhat clubbed; there was enlargement of the lower radius and ulna, and this region was distinctly tender. The changes in the lower extremities were similar to, but less marked than those in the upper. Over the thickened area, at the lower end of the tibia and fibula, tenderness was present. The subcutaneous tissue in the malar region was also thickened.

The Roentgen-ray examination showed that there was a slight increase in the soft tissues of all the fingers, most marked at the tips and around all the interphalangeal joints. Cauliflower formation was marked only at the tips of



the end phalanges of the index and little fingers. The forearms were negative. The soft tissues of all the toes were increased and the tips of the end phalanges of the big toes showed an exaggeration of a burr-like formation. The fifth metatarsal bones appeared at the inner aspect to be lined with a thin layer of new bone perfectly regular in outline and showing a sharp and distinct differentiation between it and the cortical layer. Both tibiae show on their external aspect, beginning just above the malleolus, and extending upward about 3.5 cm., a lining of a distinct, although very thin, layer of new bone formation, with complete differentiation. The same changes were found at the posterior aspect of the lower end of the tibia. At the anterior aspect there was a very fine layer of a just beginning new bone formation extending to 3.5 cm. The femora, hips and pelvis were negative.

CASE 7.—J. M., a Hungarian, aged 36 years, a tailor by occupation, showed a pulmonary condition which dated back three years. He was in the second stage of pulmonary tuberculosis, which was running an afebrile course. The sputum was positive. He had never noticed any changes in his fingers or toes, nor had he complained of pain in the joints or bones. There was present marked clubbing of the fingers and toes, also curving and thickening of the nails. The wrist and ankle joints appeared larger than one would expect in proportion to the remainder of his skeleton. The subcutaneous tissue in the malar region was thickened and the nasal tip was voluminous.

Roentgen-ray examination revealed great increase in the soft tissues of all the fingers, most marked at the tips. Very marked bony projections at the base of the end phalanges, with marked cauliflower formation at the tips. All the phalangeal bones had an abnormally short and thick appearance, the bases being greatly broadened. The forearms and arms were negative. There was marked increase in the soft tissues of all the toes and the burr-like formation at the tips of the end phalanges was quite exaggerated. The lower ends of the tibiae showed a dense layer of new bone formation extending above the malleolus upward about 5 cm., presenting in some spots a line of demarcation, in others merging with the normal cortical layer and giving the lower end of the tibia a clumsy and misshapen appearance. The fibulae failed to show any abnormality. The rest of the bones of the lower extremities were not examined.

CASE 8.—S. A., a Russian, aged 34 years, presented a condition which dated back five years. He was in the third stage of pulmonary tuberculosis. The urine was negative, the sputum positive. The patient claimed that six years before he had rheumatic pains in his arms. Four years before he noticed the changes in the ends of his fingers and toes. The fingers and toes showed marked clubbing, also curving of the nails. The nails showed striae and the matrices were thickened. There was no tenderness over any of the long bones or joints. The long bones did not show any changes. The nose was bulbous and there was a slight thickening of the subcutaneous tissue of the malar region.

The Roentgen-ray examination showed that the soft tissues of all the end phalanges were greatly increased. The basal portions of the end phalanges were broadened and showed lateral excrescences, most marked in the little fingers. The tips of the distal phalanges showed a moderate amount of burr-like projections. The rest of the bones of the hands failed to show any abnormality. The lower end of the ulna showed a thin layer of periosteal new bone, beginning just above the styloid process and extending upward about 2.5 cm., gradually merging into the normal cortex. No abnormalities were seen in the upper arms. The soft tissues of all the toes were greatly increased. The tips of the distal phalanges showed moderate cauliflower formation. Otherwise no abnormalities were seen in these bones. At the internal aspect of both tibiae there was a layer of new bone formation, beginning just above the internal malleolus and extending upward, about 10 cm. in diameter at its widest.

The external aspect of the tibia and the internal aspect of the fibula from the lower tibiofibular joint upward about 2 cm. showed marked irregularities



in outline, with many bony projections. Practically the same condition was seen on the lateral plate at the anterior and posterior regions. The anterior aspects of the femora showed a dense layer of a new bone formation about 0.5 cm. at its widest point, and extending upward about 15 cm., gradually merging into the normal cortex. There was a free space between this new bone formation and the cortex. No abnormalities were seen in the pelvic bones.

CASE 9 (No. 30729).—L. R., a Russian, aged 38 years, showed a lung condition which dated back three years. He was examined Nov. 26, 1915, and found to be in the third stage of pulmonary tuberculosis. The sputum was positive. The course of the disease was afebrile for most part, with slight variations. There was thickening of the nasal tip and of the subcutaneous tissue in the malar region. The finger nails were thickened and curved, and showed longitudinal striations. The matrices are thickened and showed slight cyanosis. There was moderate thickening of the soft tissues of the finger tips. The lower part of the forearms, in the region of the wrist, was thickened and clumsy in appearance, and this area was distinctly tender. Similar changes were not present in the legs, but the toes showed changes similar to the fingers.

Roentgen-ray examination revealed a great increase in the soft tissues of all the end phalanges and burr-like excrescences at the tips, with marked broadening of the bases of the end and middle phalanges. The outer aspect of the first metacarpal bone showed a distinct irregularity along its middle third, due to an irregularly outlined layer of periosteal new bone formation. The entire metacarpal bone appeared clumsy and considerably broadened. The lower ends of the ulna showed just above the styloid process a fine layer of new bone formation, with a distinct line of demarcation extending upward for about 4 cm. Roentgenograms made in various positions showed that the same changes were present at the dorsal aspect of the ulnae. The right radius showed a slight irregularity in the outlines just above the styloid process, extending upward about 2 cm. The upper arms were negative. There was marked cauliflower formation at the tips of the end phalanges of the toes. The fifth metatarsal bone presented at both its lateral aspects a thin layer of new bone formation, showing in some places a distinct line of demarcation. This line of demarcation disappeared entirely at the middle portions, where the entire bone showed a lack of differentiation between cortex and medulla, the latter appearing just as dense as the former. The lower ends of the tibiae just above the malleolus and extending upward about 4 cm. showed at the anterior, posterior and external aspects a layer of new bone formation with a sharp line of demarcation and varying in thickness from  $\frac{1}{4}$  to  $\frac{1}{2}$  cm., and extending upward about 4 cm. The fibulae did not show any changes. The femora and pelvic bones were negative.

CASE 10.—I. G., aged 38, a hatmaker by trade, presented an illness dating back four years. He was in the third stage of pulmonary tuberculosis, running an afebrile course. The sputum was positive. He had marked clubbing of the distal ends of the fingers and toes, with great curving of the nails. He had never had pain in any joints of the body. His nose was markedly globular, and there was a thickening of subcutaneous tissues in the malar region. The patient died Oct. 29, 1915.

The Roentgen-ray examination showed that there is marked increase in the soft tissues of all the finger tips, a moderate amount of cauliflower formation at the tips of the distal phalanges, and a quite marked broadening of the bases of the same phalanges, due to point-like excrescences most marked in the little fingers. The middle phalanges appear somewhat shortened and widened at the basal portions. There was some roentgenologic evidence of rarefaction in the upper three quarters of both ulnae and at the upper halves of the radii. In the middle portions of the ulnae there was marked decrease in the differentiation between the cortex and medulla. The upper arms were nega-

tive. There was slight irregular thickening of the cortex at the lateral and outer aspect of the middle portion of the fifth metatarsal bone on the right side and to a much lesser degree on that of the left side. There was a point-like exostosis at the lateral and outer aspect of the head of the first metatarsal bone on the right side. The rest of the bones and joints of the feet were negative. There was a layer of new bone at the lateral and inner aspect of both tibiae, beginning just above the malleoli and extending upward 5 cm. There was marked irregularity at the lateral aspects of both tibiae and fibulae along the tibiofibular joint, extending upward about 3 cm., especially along the tibiae. There was evidence of moderate amount of rarefaction in the middle and upper portion of the right fibula.

The pathologic report gave an anatomic diagnosis of chronic ulcerative pulmonary tuberculosis, tuberculosis laryngitis and tracheitis with ulceration, tuberculous ulcer of the rectum, miliary tubercles of the lungs, chronic adhesive pleuritis, ossified periostitis, chronic adhesive pericarditis, atheromatous change in pulmonary arteries, dilatation and hypertrophy of right auricle and ventricle, edema of the skin and mesentery, ascites, bilateral hydrothorax, chronic passive congestion of the liver, thrombosis of left suprarenal vein and of a branch of the left pulmonary artery.

Microscopically a section of the various bones (fibula, tibia, radius, ulna, fifth metatarsal, terminal phalanx, middle finger, in the areas corresponding to Roentgen-ray changes) revealed a constant abnormality. There were masses of new bone within the periosteum, separating it from the old bone. This new bone was irregular in distribution and was composed of numerous osteoblasts imbedded in a homogeneous matrix, similar to the old bone in general, but not so prominently laminated. The bone marrow was fatty; the process was best seen in the lower end of the radius and tibia. A section of the lower end of the radius showed the periosteum invaded by irregular masses of new bone. This new bone was irregular in distribution and was composed of numerous osteoblasts embedded in a homogeneous matrix similar to the old bone. It likewise showed lamination, not, however, as prominently as the older bone. This ossifying process was seen in all portions of the periosteum in the section. At the lower end of the tibia the periosteum everywhere showed a similar ossifying process to that described above.

I wish to express my thanks and appreciation to Dr. Scholz of the Roentgen-ray Department at Montefiore Hospital for his careful work done in this connection.

## TYPHOIDIN QUOTIENTS

### AN ANALYSIS OF THE FACTORS OF UNCERTAINTY IN THE CUTANEOUS TYPHOIDIN TEST \*

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In connection with a recent study<sup>1</sup> of the cutaneous typhoidin test, which was introduced by Gay and Force<sup>2</sup> as an index of typhoid immunity, an objective method of reading such cutaneous diagnostic reactions was described. Instead of reporting the test as positive, negative or doubtful, from the general appearance of the inoculated spots, the diameters of the areolae are measured with a suitable millimeter gage, preferably one which can be set to the diameter to be measured without exhibiting the reading until afterward. The result of a test is recorded as the quotient of the diameter of the test areola divided by that of the control. The advantages of this procedure are, first, that after adjusting the gage to the diameters of the areolae, the bias of the observer can be entirely eliminated in expressing the results of the test, and, second, that the quotients so obtained lend themselves readily to quantitative analyses according to the statistical theory of variables. Reasons were given for using the quotient rather than the difference between the two diameters. It is recognized, of course, that other characters, such as swelling, induration and depth of color, may contribute to the formation of judgments concerning such diagnostic skin reactions. As a rule, however, these characters vary with the diameters; and for statistical investigations, it is felt that the advantages of the definite and uniform system of measurements possible for the diameters outweigh any objections to the ignoring of other qualities of the reactions.

It was found that with the dry preparation of typhoidin used there were average differences between groups of typhoid immunes and nonimmunes, but that the differences were small in comparison with the variation among individuals in the same group, so that the test had little value for the individual case. The desirability, therefore, became apparent of examining possible sources of variability in

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\* From the Department of Medicine of the University of California Medical School.

1. Kilgore, E. S.: The Typhoidin Quotient, *THE ARCHIVES INT. MED.*, 1916, **17**, 25.

2. Gay, Frederick P., and Force, John N.: A Skin Reaction Indicative of Immunity Against Typhoid Fever, *THE ARCHIVES INT. MED.*, 1914, **13**, 471.



the results of the test other than differences in typhoid immunity. Also, the dry typhoidin had been substituted for the aqueous solution previously used, because it was thought that the latter deteriorated rapidly.<sup>3</sup> The question now should be answered whether or not the dry powder, possibly because it is slightly hygroscopic, loses potency on standing.

In order to determine the sources of variability of the test other than differences in immunity, the personal equation in making the measurements has been investigated, as well as variations in other factors, such as the mechanical trauma and the amount of the applications absorbed. In order to test the keeping qualities of the dry typhoidin an old preparation of it has been compared with a new preparation on a new series of cases. At the same time advantage was taken of the opportunity to compare several different preparations of typhoidin.

The technic was the ordinary Pirquet technic heretofore described.<sup>1</sup>

#### SOURCES OF ERROR IN THE APPLICATION AND READING OF THE TYPHOIDIN CUTANEOUS TEST

*The Personal Equation of the Observer in Measuring the Areolae.*—In order to determine the variation to be expected among these cutaneous reaction determinations on account of the personal factor of the observers alone, a pair of one-day old areolae (one spot inoculated with "T<sub>4</sub> (new)" and the other with the corresponding control powder, described later, were measured successively forty-one times by as many different persons (twenty-nine physicians and twelve medical students) in the University Hospital, all the measurements being made in one forenoon and under the same conditions of light as nearly as possible. The subjects of the experiment used a pair of draftsman's compasses and the measurements therefrom were then transferred to a millimeter scale. Possibly on account of the exposure of the arm, during the first few measurements both areolae seemed to increase somewhat in diameter, according to both my own measurements and those of the others. The measurements of the first six subjects were therefore thrown out, and the results of thirty-five only are included in Table 1.

Although each person was instructed to set the calipers to the extreme limits of the areolae which he could see, it is apparent from the table that there was great variation among the measurements by different ones; and this is inevitable from the fact that these areolae are not sharply limited at the periphery, but fade gradually. The

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3. Gay, Frederick P., and Claypole, Edith J.: An Experimental Study of Methods of Prophylactic Immunization Against Typhoid Fever, *THE ARCHIVES INT. MED.*, 1914, **14**, 671.

TABLE 1.—MEASUREMENTS OF ONE PAIR OF AREOLAE BY THIRTY-FIVE PERSONS DURING ONE FORENOON

	Mean	Probable* Error	Lowest	Highest	Theoretical Limits*
Test spot.....	21.2 mm.	$\pm 2.64$ mm.	14.8 mm.	29.4 mm.	10.91 to 31.49 mm.
Control.....	6.52 mm.	$\pm 0.85$ mm.	4.2 mm.	9.2 mm.	2.76 to 10.28 mm.
Typhoidin quotient.....	3.31	$\pm 0.4$	2.45	5	1.51 to 5.11

\* The probable error was obtained according to the formula,

$$E = \pm 0.6745 \times \text{the standard deviation}$$

(Davenport: "Statistical Methods with Special Reference to Biological Variations," John Wiley and Sons, New York, 1914). A probable error of  $\pm 2.64$  mm. for measurements of the test spot means that if one of the readings in the series is picked out at random, the chances are even that it will differ by more or by less than this amount from the mean.

The standard deviation of the test spot measurements is 3.76 mm. according to the formula:

$$\text{Standard deviation} = \sqrt{\frac{\text{Sum of the squares of all deviations from the mean}}{\text{Number of variates}}}$$

It is an interesting fact that in a frequency distribution of the symmetrical or nearly symmetrical type, a range of six times the standard deviation includes about 99 to 100 per cent. of all the values (G. U. Yule: "Introduction to the Theory of Statistics," Charles Griffin & Co., London). That is, in the present case, assuming the distribution to be symmetrical, three times the standard deviation subtracted from the average ( $21.20 - 3 \times 3.76 = 10.91$ ) would represent approximately the lower limit, and the same amount added to the average ( $21.20 + 3 \times 3.76 = 31.49$ ), approximately the upper limit of measurements of these areolae if the series were extended to include observations by many more persons of the same general character as these thirty-five. The distribution in all these cases, however, is more or less askew, and the series so small that these theoretical limits are quite rough. It will be seen, however, that they are not very far from the actual highest and lowest figures recorded.

greatest disagreement (largest probable error) is seen in the measurements of the typhoidin areola. One man will detect a faint blush 29 mm. in diameter, while another sees about half as much. Fortunately, the one who makes the small measurement of the test spot also, as a rule, makes a conservative determination of the control areola, so that the quotients show proportionately less variation than either the dividends or divisors, a fact which emphasizes the utility of control inoculations in such tests.

It should be stated here, however, that while the test areola in this case was judged to be rather more ambiguous than the average, it was less so than some; so that the comparison between control and test spot is not always so effective in limiting the personal equation as is shown in these figures. In typhoidin, tuberculin and other similar cutaneous tests there is at times a quite distinct small, fairly sharply limited areola, surrounded by a much wider but faint and gradually fading blush. The control spot may have the same double character, but as a rule in such cases it is of the ordinary type. If such a pair of areolae were submitted to different observers for measurement, the resulting quotients would certainly be more at variance than is the case in this series. In order to be consistent throughout it is our prac-

tice always to measure the diameter of the outermost blush that can be seen. But in so doing it is recognized that the measurements probably do not always represent corresponding degrees of the same biologic process. For all gradations of the double areola described above are encountered, from the well-marked outer blush to one which is scarcely visible; and there is no doubt that among the cases in which but one areola is observed are some which correspond to the inner brighter areola of the double type. That is, it may well be that the faint outer blush is really present, but is concealed in these cases by the heavier epidermis, or that it involves a deeper set of capillaries so that it escapes observation and measurement. It is also true, however, that the cases with the double areola, and hence with the high typhoidin quotients, are the ones which all would agree showed a very "strong reaction."

In the investigation of a series of cases (to which, as has already been intimated, the use of the test should be confined) it is reasonable to expect that if the measurements were all made by one observer they would be more consistent. To determine the variation among my own readings I measured during the same forenoon the same two areolae described above once for each measurement by the other observers. Omitting, as before, the first six figures, the results for the last thirty-five are shown in Table 2.

TABLE 2.—MEASUREMENTS OF ONE PAIR OF AREOLAE THIRTY-FIVE TIMES BY ONE PERSON DURING ONE FORENOON

	Mean	Probable Error	Lowest	Highest	Theoretical Limits
Test spot.....	21.9 mm.	$\pm 1.07$ mm.	19 mm.	25.2 mm.	17.2 to 26.7 mm.
Control.....	6.03 mm.	$\pm 0.42$ mm.	5 mm.	7.5 mm.	4.2 to 7.9 mm.
Typhoidin quotient.....	3.65	$\pm 0.28$	3	4.82	2.4 to 4.9

The probable errors, which may be taken as measures of the inconsistency in these readings, are about half as great in Table 2 as in Table 1. *In other words, about half the uncertainty connected with these measurements is due to differences in the separate standards of observers, and can be eliminated in a series of observations if they are all made by one worker.*

That the discrepancy among these measurements is largely due to causes which vary from hour to hour is shown by the considerably better results when I measured a similar pair of areolae an equal number of times but in quick succession during a half hour. These results are shown in Table 3.



TABLE 3.—MEASUREMENTS OF ONE PAIR OF AREOLAE THIRTY-FIVE TIMES BY ONE PERSON DURING A HALF HOUR

	Mean	Probable Error	Lowest	Highest	Theoretical Limits
Test spot.....	16.5 mm.	$\pm 0.7$ mm.	13.3 mm.	18.5 mm.	13.1 to 19.9 mm.
Control.....	4.6 mm.	$\pm 0.15$ mm.	4.1 mm.	5 mm.	3.9 to 5.3 mm.
Typhoidin quotient.....	3.6	$\pm 0.19$	3.12	4.28	2.75 to 4.46

Although the areolae in this case resembled in every way the ones in the previous experiment, it will be noted that the probable errors are only about one third to two thirds as large as before. The differences between Tables 2 and 3 are important in connection with the interpretation of cutaneous reactions; and the larger discrepancies of Table 2 are of course the ones which correspond more closely to the conditions of actual practice in gathering statistics from a series of cases. *That is, the results of reading these cutaneous tests depends considerably on some such conditions as the hour of the day, perhaps the atmospheric temperature, and the activity of the subject, as well as possibly on unavoidable variation in the way in which the observer exercises his faculties.*

*Variations in the Typhoidin Cutaneous Reaction which are Independent of Allergic Differences.*—After allowing for inaccuracies in the readings, it is certain that not all of the variations in the size of these inoculation areolae are due to allergic mechanisms of any sort. Variations in the strain of the organism used, materials in the culture mediums, methods of preparation, etc., may affect the results, as will be shown later. But in addition to these variables, which can be standardized for a given series of observations, there are others inherent in the method. Thus, the amount of typhoidin powder used in each test, even if it could be accurately measured, is no indication of the amount absorbed. In nearby areas the skin may vary in glandular content, hair follicles or otherwise; but perhaps the most variable factor present is the amount of trauma inflicted by the chisel, even when the greatest care is taken to standardize the technic.

To test the effect of these variables, Dr. E. H. Falconer was good enough to make 100 circular abrasions on the front of my thigh, by rotating a chisel with a 2.5 mm. blade. His aim was to make them all as nearly as possible alike and just deep enough not to cause bleeding. Half of them were then inoculated with typhoidin powder "T<sub>4</sub> (new)" (described later), the other half were left to show the effect of the mechanical trauma alone. On the following day I measured the transverse diameters of all the 100 areolae, using the technic already described to avoid being biased by seeing the measurements during the act of taking them. In the order in which the abrasions were made the

areolae were divided into pairs, one test spot and one control, for the purpose of forming typhoidin quotients.

On inspection of both the control and the inoculated spots a certain amount of variation was very evident, which is reflected in the results of the measurements shown in Table 4.

TABLE 4.—MEASUREMENTS OF FIFTY PAIRS OF AREOLAE ON ONE SUBJECT MADE BY ONE OBSERVER DURING ONE-HALF HOUR

	Mean	Probable Error	Lowest	Highest	Theoretical Limits
Control.....	4.97 mm.	0.38 mm.	3.7 mm.	6.3 mm.	3.29 to 6.65 mm.
Typhoidin.....	10.16 mm.	0.87 mm.	7 mm.	12.6 mm.	6.31 to 14.01 mm.
Quotient.....	2.07	0.24	1.25	2.72	1.03 to 3.11
Possible quotient*.....	.....	.....	1.11	3.4	

\* Obtained respectively by dividing the greatest test spot diameter by the smallest control and vice versa.

As these measurements were made in quick succession, they are to be compared with Table 3 to show the added influence of variations connected with the application of the test. The fact that the greatest difference between the probable errors in the two tables happens in the case of the uninoculated control spots suggests that much of the variation comes through the inability of the operator always to produce the same amount of trauma. It should be stated, however, that the control abrasions were made before those intended for inoculation, so that there was opportunity for the operator to better the standardization of his technic by the time he made the second fifty abrasions.

#### PREPARATIONS OF TYPHOIDIN POWDER

In order to test the keeping qualities of typhoidin powder and also to compare several preparations of typhoidin, a new series of tests was undertaken on 103 students, nurses, physicians and others connected with the University of California Hospital and the Hooper Foundation for Medical Research. By using the ordinary Pirquet technic as before, nine circular abrasions were made on the arm of each subject, making them all as nearly as possible equal in depth (just short of bleeding) and on skin of as nearly as possible the same texture. One spot was not inoculated, and was for the purpose of showing the effects of the mechanical trauma alone. On each of the other eight spots was rubbed a little of one of the following preparations:

1. "T<sub>2</sub> (old)": This typhoidin was described in a former communication.<sup>1</sup> It was prepared about eighteen or twenty months previously in the department of pathology and bacteriology of the University of California, and had been kept meantime in a rubber-corked, amber-colored bottle in the open laboratory.



2. "C<sub>2</sub> (old)": This was the control application, the manufacture, preservation and use of which were the same as for "T<sub>2</sub> (old)."

The other preparations, which follow, were kindly furnished by Dr. Karl Meyer of the George Williams Hooper Foundation for Medical Research of the University of California. Their manufacture was completed only a few hours before beginning these experiments, and they were kept in amber-colored bottles in a desiccator. A detailed description of methods of preparation is to be given in a forthcoming article by Dr. Meyer, and the following statements will suffice here:

3. "T<sub>4</sub> (new)": This was prepared from the same strain (Dorset) and in the same way (using Witte's peptone) as "T<sub>2</sub> (old)."

4. "C<sub>4</sub>": This was the corresponding control powder.

5. "T<sub>7</sub>": This powder was prepared from eight strains, known as "Sch., B., L., Kuhn, 66, 94, 38 and M." Liverbroth and Witte's peptone were used. Hence "C<sub>4</sub>" also serves as control for this typhoidin.

6. "T<sub>8</sub>": This powder was prepared from Rawlings strain of *Bacillus typhosus*, using Chapoteaut-Paris peptone.

7. "C<sub>8</sub>": This was the corresponding control powder.

8. "T<sub>2</sub>": This was the Dorset strain of *B. typhosus*. Chapoteaut peptone was used, so that the corresponding control powder is "C<sub>8</sub>."

About twenty-four hours after inoculation the diameters of all the areolae were measured in a direction across the arm. A summary of the results is expressed in Table 5. The measurements of the uninoculated spots are in millimeters. In order to show the relative amount of added reaction about the spots which were inoculated with the control powders, the diameter of each was divided by that of the uninoculated spot and these quotients are shown in the tables: The typhoidin inoculated abrasions, on the other hand, are to be interpreted according as they exceed the reactions about the spots inoculated with the corresponding control powders; and they are, therefore, tabulated as the quotients of the test spot diameter divided by the control spot diameter, that is, as "typhoidin quotients." In each case the first figure given is the mean for the corresponding group, and the "plus or minus" figure below it is the probable error of the mean.<sup>4</sup>

4. This was obtained (Davenport, Footnote to Table 1) according to the formula:

$$\text{Probable error of the mean} = \pm 0.6745 \sqrt{\frac{S}{n^3}}$$

In the larger groups, like the group of immunes, which is composed of the subgroups A, B and C, the probable errors were first computed for the subgroups; and from these figures (that is, by adding the *S*'s and the *n*'s) the probable errors for the composite groups were estimated. Since the averages for the subgroups were not quite identical, the probable errors of the large groups computed in this way may not be quite the same as if an entire, new calculation had been undertaken in each case. It is certain, however, that whatever inaccuracies may be present from this cause are small in comparison with the probable inaccuracies of the probable errors themselves, and are therefore inconsequential.



TABLE 5.—RESULTS OF TYPHOIDIN TESTS ON A SERIES OF CASES

Group	Classification of Subjects	Uninoculated Areola, Mm.	Control Inoculations			Typhoidin Quotient					
			C <sub>2</sub> (old)	O <sub>4</sub> (new)	C <sub>3</sub>	T <sub>2</sub> (old)	T <sub>4</sub> (new)	T <sub>7</sub>	T <sub>3</sub>	T <sub>2</sub>	Aver. T <sub>1</sub>
A	17 who gave history of typhoid fever	4.2 ±0.11	1.33 ±0.09	1.13 ±0.04	1.45 ±0.09	1.53 ±0.07	1.65 ±0.07	1.28 ±0.03	1.44 ±0.07	1.6 ±0.06	1.5 ±0.04
B	23 who had had army vaccine	3.99 ±0.07	1.25 ±0.03	1.13 ±0.04	1.23 ±0.03	1.38 ±0.03	1.51 ±0.04	1.35 ±0.04	1.42 ±0.05	1.42 ±0.04	1.41 ±0.03
C	38 who had had sensitized vaccine	4.11 ±0.08	1.19 ±0.02	1.1 ±0.02	1.2 ±0.02	1.51 ±0.05	1.57 ±0.04	1.28 ±0.04	1.4 ±0.04	1.6 ±0.04	1.47 ±0.03
D	78 who had had typhoid* or vaccine* of either kind	4.1 ±0.05	1.18 ±0.02	1.07 ±0.01	1.2 ±0.02	1.51 ±0.03	1.6 ±0.02	1.33 ±0.02	1.45 ±0.03	1.57 ±0.03	1.46 ±0.01
E	23 who had had neither typhoid nor vaccine	3.81 ±0.07	1.32 ±0.05	1.17 ±0.03	1.26 ±0.05	1.43 ±0.05	1.45 ±0.05	1.3 ±0.04	1.41 ±0.05	1.48 ±0.02	1.41 ±0.04
F	32 who had had but one course of immunization	4.2 ±0.05	1.16 ±0.02	1.12 ±0.03	1.17 ±0.03	1.39 ±0.03	1.51 ±0.04	1.31 ±0.03	1.4 ±0.05	1.52 ±0.05	1.42 ±0.03
G	29 who had had more than one course of immunization	3.9 ±0.06	1.26 ±0.03	1.11 ±0.02	1.25 ±0.02	1.55 ±0.06	1.58 ±0.04	1.31 ±0.04	1.42 ±0.04	1.53 ±0.04	1.43 ±0.03
H	General averages for all subjects	3.88 ±0.04	1.2 ±0.02	1.09 ±0.02	1.21 ±0.02	1.49 ±0.03	1.57 ±0.02	1.33 ±0.03	1.44 ±0.03	1.55 ±0.03	1.45 ±0.02

\* History of typhoid fever at any time or antityphoid vaccination within the preceding ten months.

The first column in Table 5 shows the averages of measurements of the uninoculated spots. The lowest average, 3.81 mm., is in the group with negative history, the highest, 4.2 mm., is in the group of those who had had one course of typhoid immunization and also in the typhoid group. The suggestion from these averages is that those who have had typhoid fever or one course of vaccination tend to react more than others to mechanical trauma of the skin. Such a conclusion should never be drawn from averages, however, without first taking into consideration the number of the variables from which the averages are drawn and the amount of variation among them, and accordingly the possibility that the differences observed between the averages may be the result merely of "fluctuations of sampling." The amount of variation (relative to the number of observations) among the variables

underlying these two averages is reflected in their probable errors, 0.11 and 0.07 mm. According to the formula,

$$\text{Probable difference of } A_1 \text{ and } A_2 = \sqrt{E_1^2 + E_2^2}$$

the probable difference between these two averages is

$$\sqrt{0.11^2 + 0.07^2} = 0.13 \text{ mm.}^5$$

This means that if there is really no difference between these two classes of persons in their tendency to react to mechanical trauma, then if the work were repeated on equal numbers of similar cases, the chances would be equal that the differences between the two averages would be greater or less than 0.13 mm. The actual difference between the two averages here considered is 0.39 mm., or three times the probable difference. It can be shown<sup>6</sup> that there is about one chance in twenty-one that two averages as far apart as these have been derived from the same kind of material, that is, that the differences are due to errors of sampling alone. This would lend a very fair degree of probability to the idea that a real difference in the tendency to react to cutaneous trauma exists between those who have had typhoid fever and those who have not, if it were not for the fact that the two averages 4.2 and 3.81 were selected for comparison because they are respectively the highest and the lowest among the seven figures under consideration. A simple calculation shows that if two out of these seven averages were picked without selection there is exactly one chance in twenty-one that these particular two averages would have been taken. The conclusion, therefore, is that the difference between these averages is exactly what should be expected to result solely from the variability in the material; in fact, they are nearer the variations theoretically to be expected from "errors of sampling" than one would have any right to anticipate.

There is, of course, no reason to expect that one class of these subjects would react more to simple trauma than another class; and these average reactions have been dwelt on because they illustrate so well the mistakes frequently made, particularly in medical literature, in interpreting averages. Differences which superficially seem very striking and significant may often, as in this case, be shown to be entirely devoid of meaning, if the trouble is taken to examine in the manner here illustrated the material from which they are derived.

As might be expected, similar calculations show that among the reactions to the various control powders the differences between the

5. "The probable difference between two averages,  $A_1$  and  $A_2$ , of which the probable errors  $E_1$  and  $E_2$  are known, is the square root of the sum of the squared probable errors" (Davenport, Footnote to Table 1).

6. Davenport, Footnote to Table 1.

averages for different groups of individuals, though at first suggestive of differences between the groups, are not large enough in relation to their probable errors to be at all significant. Some of the greatest differences, moreover, are between groups D and E; and, since these average quotients depend on the measurements of the uninoculated spots, which formed the divisors, the average difference between these measurements in these two groups practically accounts for the differences between the quotients. The general averages for all groups (*H*) show only minor differences between the control powders, which are easily chargeable to fluctuations of sampling.

Unfortunately for the usefulness of the typhoidin test, the typhoidin quotients (last five columns of Table 5) show no greater relative group differences than the control reactions or the uninoculated spots. It would have to be admitted, therefore, that these results were well within the limits of experimental error and might be entirely without significance of typhoid immunity if it were not for the fact that the results with the different preparations agree among themselves and with those of a previous series<sup>1</sup> as well as with the observations of other observers.<sup>2</sup> A further suggestion that the typhoidin test bears some relation to typhoid immunity is found in a comparison of groups F and G. Those who have had two or more courses of typhoid immunization show slightly higher quotients than those who have had but one course; and this is true with all the preparations of typhoidin except "*T*<sub>7</sub>," in which case the two averages are equal.

The different preparations of typhoidin show only minor differences in the results. The old preparation, "*T*<sub>2</sub> (old)," shows slightly but only slightly less contrast between the groups of immunes and non-immunes than the corresponding "*T*<sub>4</sub> (new)," which was freshly prepared; and the general average in all classes was a little greater with the new preparation. If these averages were based on thousands of observations instead of on one hundred, they would undoubtedly indicate that over a period of two years the dried typhoidin loses a small part of a weak ability it had at the outset to call forth a specific reaction, and that it also loses some of its original nonspecific toxicity. The figures as they stand, showing small contrasts in proportion to their probable errors, can only be interpreted as suggesting that typhoidin undergoes these changes with age. It can be said quite definitely, however, that the changes with age are not great.

As to the other preparations of typhoidin which were tried, "*T*<sub>7</sub>" (mixed strains, Witte's peptone), "*T*<sub>3</sub>" (Rawlings' strain, Chapoteaut peptone), "*T*<sub>2</sub>" (Dorset strain, Chapoteaut peptone), it will be seen by comparing groups D and E and groups F and G in Table 5 that so far as these figures can be relied on, these preparations appear to be inferior to "*T*<sub>4</sub> (new)," which was prepared from Dorset strain,



Witte's peptone being used. They show less contrast between immunes and nonimmunes and between those who had had but one course of vaccination and those who had had more than one course.

The last row (H) in Table 5, which shows the general averages for all the groups, is interesting in that it exhibits independently of specific differences among the groups of individuals (which differences have been shown to play a very minor rôle in the total reaction) the approximate proportions of these cutaneous reactions which are due to individual factors. Mechanical trauma alone is accountable for an areola with average diameter of 3.88 mm., that is, 1.38 mm. in excess of the width of the chisel. The material in the control powder increases the diameter of the area of redness by from 9 to 21 per cent., and the material from the typhoid organisms, together with that from the culture mediums, produces an additional 33 to 57 per cent. increase over the control inoculation. Compared with this, 33 to 57 per cent. average difference between the control and the typhoidin spot diameters is less than 15 per cent. difference between the average typhoidin quotients of immunes and nonimmunes. *In other words, what may be termed the nonspecific toxicity of typhoidin has three or more times as much influence on the results of the reaction as any specific differences among the subjects related to their previous experience with typhoid infection or vaccination.*

This nonspecific toxicity may be due to the material of the typhoid organisms themselves or to bodies which they have split off from the proteins of the culture mediums. Whatever substances are responsible for it, it seems clear that this nonspecific toxicity, itself undoubtedly quite variable in its effects on different persons, is the thing that almost completely overshadows the specific part of the reaction and makes impossible any reliance on the test for individual cases. And until some way is found of very much reducing this disturbing element in the reaction (possibly by changes in the technic of preparation of the typhoidin or by adopting some other criterion for determining the results than the diameters of the twenty-four-hour areolae), little can be hoped from any use of the test. It must be remembered, also, that even if this alteration were accomplished, more evidence would still be needed to show that the specific reaction was definitely connected in a quantitative way with immunity to typhoid fever, and that the two things were not simply usually or occasionally associated.

#### INTRACUTANEOUS TYPHOIDIN TESTS

So far the intracutaneous technic has not proved itself better for typhoidin<sup>7</sup> than the Pirquet cutaneous method introduced by Gay and

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7. Austrian and Bloomfield: THE ARCHIVES INT. MED., 1916, **17**, 663.

Force. Our own experience with it was entirely disappointing, since in a series of ninety-one cases the reactions after twenty-four hours were just as pronounced (if anything a little more so) among those with negative history as among the groups which had had typhoid fever or prophylactic inoculations. Our experiments, while not conclusive, in that only one strength of typhoidin solution was used for the injections (1 to 100 of "T<sub>4</sub> (new)," evidently a much too strong solution) and only the diameters of the twenty-four-hour areolae were measured, left us with the feeling that little is to be expected from typhoidin applied by any technic unless some way can be found to overcome the large nonspecific reaction which it produces. The suggestion of Pulay<sup>8</sup> to interpret the reactions according to their persistence rather than the diameters of the areolae should be further investigated; and work to this end is soon to be reported by Force from the department of hygiene of this university.<sup>9</sup>

#### COMMENT AND SUMMARY

The facts brought out in this study indicate even more strongly than those formerly reported<sup>1</sup> that the cutaneous typhoidin test should not be relied on as an index of immunity in individual cases, and that even in a study of groups of cases its results need to be considered with much conservatism. The use of "typhoidin quotients" and of statistical methods of analyzing them, while throwing light on the shortcomings of the typhoidin test, are in no way responsible for them, for although the uncertainties in measuring the areolae, which have been analyzed in this report, have been found to be very considerable, there is no reason for supposing that they are greater in any way than the uncertainties involved in simply judging the result of a skin test. After the measurements are made, the mathematical methods of dealing with them (as previously explained<sup>1</sup>) are practically identical with the subconscious methods involved in the formation of judgments as to the results of the test, but are of course free from all further vagaries involved in such judgments. It is again urged that similar methods of study should be applied to other cutaneous tests in order to ascertain much more definitely the amount of reliance which can be placed on them.

These studies concerning the amount of inaccuracies in reading the cutaneous typhoidin reactions may be presumed to represent very nearly the conditions which exist for other similar cutaneous diagnostic tests, and may be summarized as follows: When a single pair of areolae were measured many times in rapid succession by one individual, the resulting typhoidin quotients have a probable error of 0.19,

8. Pulay, E.: *Wien. klin. Wchnschr.*, 1915, **28**, 1189.

9. Force, J. N., and Stevens, Ida M.: *Further Studies on Typhoidin*, *THE ARCHIVES INT. MED.*, to be published.

or about 5 per cent., of the general average of the quotients. When the same measurements are made by the same individual during the course of a forenoon, changes either in the observer or in the areolae or both result in a probable error among the quotients of 0.28, or about 8 per cent. This represents most nearly the amount of variation to be expected from measurement errors in a series of cases observed by one person. If the readings of a number of observers made on one pair of areolae during one forenoon are compared, the probable deviation of the typhoidin quotients from the average of the group is 0.4, or 12 per cent. of the average. In addition to variations in measuring the areolae, other discrepancies are connected with the application of the trauma and the inoculations. A rapid series of readings by one observer of a number of tests applied on the same subject yielded a probable error of the quotients of 0.24, or about 11 per cent. of the average quotient. The difference between this result and the 5 per cent. when the same experiment was performed with one pair of areolae is 6 per cent.

In other words, each of the following factors contributes to the inaccuracies in the readings in about the proportion given:

1. Indefiniteness in areola outlines, 5 per cent.
2. Effect of scattering the readings over several hours, 3 per cent.
3. Effect of participation by many observers, 4 per cent.
4. Effect of variations in the trauma, etc., 6 per cent.

#### CONCLUSIONS

1. A second series of cases has emphasized the total unreliability of the cutaneous typhoidin test in its present state of development as an index of typhoid immunity in persons and the need for much caution in interpreting its results even in a considerable series of cases. The reasons for this unreliability appear to be, first, unavoidable variations in the application of the test; second, indefiniteness of the readings, and, third, and most important, the relatively large amount of nonspecific reaction which is produced by typhoidin—upward of three times the amount that can be attributed to specific differences among the subjects.

2. In varying degrees, similar sources of inaccuracy undoubtedly exist for other cutaneous tests, and should be investigated.

3. The dried typhoidin is little affected by age.

4. The typhoidin test when applied intracutaneously in a dilution of 1 to 100 and read twenty-four hours later is valueless.



## A COMPARISON OF TWO METHODS OF VACCINATING AGAINST TYPHOID FEVER\*

EUGENE S. KILGORE

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Of the fifteen or twenty recognized methods of immunizing against typhoid fever,<sup>1</sup> the one most commonly used in this country has been that employed in the United States Army, which consists of three injections at seven or ten day intervals, the first of 500 million and the second and third of 1 billion each of a heat-killed culture of a mild strain of typhoid bacillus. Another method which has been extensively used in California in the last three years is that introduced by Gay and Claypole.<sup>2</sup> This vaccine consists of alcohol-killed cultures of the typhoid organism which have previously been treated by an immune serum, a so-called sensitized vaccine. The cultures are then dried and ground, suspended in salt solution, and the sediment redried, and weighed amounts are used in immunizing. Experimentally in rabbits such a vaccine was thought to produce more permanent immunity than nonsensitized vaccine, and it was expected to give fewer unpleasant symptoms following human inoculation.

In several hundred vaccinations of university students, Force<sup>3</sup> was convinced that the reactions were milder than with the vaccine previously used; and our experience in the University Hospital led us to the same belief. Thus, in the summer of 1912, sixty members of the University of California Hospital staff were vaccinated according to the United States Army plan with vaccine obtained through the courtesy of the Letterman General Hospital. The next year about seventy new subjects were vaccinated with sensitized vaccine according to the three-day interval plan, the vaccine being prepared in the University of California department of pathology and bacteriology under the direction of Professor Gay. Both years the attempt was made to get

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\* From the Department of Medicine of the University of California Medical School.

1. Metchnikoff and Besredka: *Ann. de l'Inst. Pasteur*, 1911, **25**, 193. Friedberger, in Kraus and Levaditi: *Handbuch der Technik und Methode der Immunitätsforschung*, Fischer, 1908, **1**, 722. Fornet, in Kolle and Wassermann: *Handbuch der pathologische Mikroorganismen*, Ed. 2, Fischer, 1912, **3**, 837. Gay and Claypole: *THE ARCHIVES INT. MED.*, 1914, **14**, 671.

2. Gay and Claypole: Footnote 1.

3. Force: *Am. Jour. Pub. Health*, 1913, **3**, 750.

reports of symptoms and temperatures following injections, and the following comparison is shown by the records:

	Army Vaccine (1912)		Sensitized Vaccine (1913)	
	Per Cent.	No. Reports	Per Cent.	No. Reports
Local Reaction				
Slight or zero.....	86.6	157	99.4	172
Moderate .....	9.4	17	0.6	1
Severe .....	4.0	7	0.0	0
	100	181	100	173
General Reaction				
Slight or zero.....	94.0	160	99.3	140
Severe .....	6.0	10	0.7	1
	100	170	100	141
Highest Temperature				
36.5 to 37.4 C.....	93.8	167	97.6	167
37.5 to 38.4 C.....	4.5	8	1.2	2
38.5 to 39.5 C.....	1.7	3	1.2	2
	100	178	100	171

This comparison, while speaking for the milder reactions following inoculation of the sensitized vaccine, was not considered conclusive for the reason that the conditions were not exactly the same both years. A greater proportion of the subjects in 1913 were men, and at that time the word was out that the reactions were expected to be milder than the year before. Moreover, the dosage of the sensitized vaccine, according to the method of preparation at that time, amounted to about one-third less than the dosage now employed, that is, it was approximately 500 million to the cubic centimeter.

Last year, therefore, at the suggestion of Professor Gay and Dr. W. A. Sawyer,<sup>4</sup> director of the State Hygienic Laboratory, it was decided to reinvestigate the subject of reaction severity for the two methods under exactly parallel conditions; and also to attempt a measurement of the relative efficiency of the two types of vaccination, as gaged by the cutaneous typhoidin test.

The army vaccine used was a fresh supply from Washington obtained through the courtesy of Capt. Henry J. Nichols of the Letterman General Hospital. It was administered within about six to eight weeks after the date of its manufacture.<sup>5</sup> The sensitized vaccine

4. Since this work was begun an important report has been contributed by Sawyer (Jour. Am. Med. Assn., 1915, **65**, 1413), who collected from physicians in California data concerning 4,967 persons vaccinated with sensitized vaccine and 2,906 who had been given other vaccines of the general type corresponding to the Army and Navy preparation. Contrary to expectations, the average clinical impressions concerning reaction severity showed no decided difference between the two types of preparation.

5. Nichols (Jour. Exper. Med., 1915, **22**, 780) has reported an apparent relation between the age of the vaccine and its toxicity.

was that supplied by the State Hygienic Laboratory. This was also a fresh preparation, the manufacture of which was completed from six to ten weeks before administration. Care was taken to shake the vaccine before administration, and the usual precautions against vaccinating during menstruation or illness were observed. The injections were made subcutaneously about the insertion of the deltoid. The subjects consisted of nineteen nurses, twenty medical students, twenty-seven members of the staff and help in the University Hospital, and

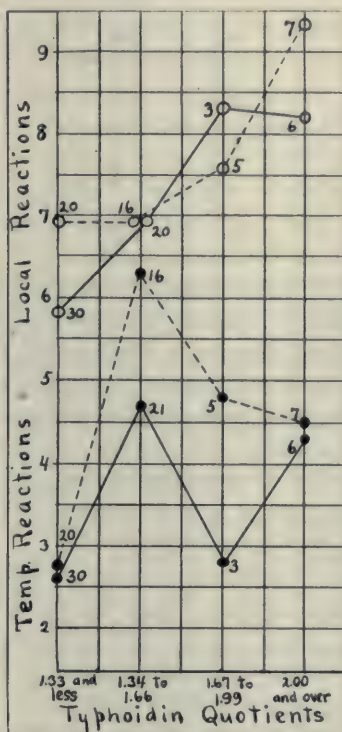


Fig. 1.—The relation between the severity of the local and general reactions following antityphoid inoculation to the previous typhoidin quotients of the subjects. The dots indicate temperature reactions, circles local reactions, solid lines those who received army vaccine, and broken lines those who received sensitized vaccine. At the bottom are indicated the grades of typhoidin quotients; the numbers at the left indicate tenths of a degree centigrade above 37 for the temperature reactions and also grades of local reactions according to the formula in the text. Numbers connected with the dots and circles indicate numbers of individuals on which the corresponding average reactions are based.

twenty dental and pharmaceutical students; fifty-three were men, thirty-two women, eighty-five in all, most of whom were young adults, a few middle aged.

Since there are two chief differences between the two methods



(preparation and dosage of the vaccine, and interval of administration) the subjects were divided into four groups, as follows: Group 1 received army vaccine at two-day intervals; Group 2 the same at ten-day intervals; Group 3 received sensitized vaccine at two-day intervals; and Group 4 the same at ten-day intervals.

The dosage for the army vaccine was always 0.5 c.c. (500 million) for the first dose and 1 c.c. for each of the other two doses; and for the sensitized vaccine 1 c.c. (equivalent to about 750 million) at each of the three doses. These unequal doses of the two vaccines were given because it was desired to compare the methods as they are being used.

Care was taken in forming the groups to have them all as equal as possible both in numbers and in composition, that is, containing approximately the same percentage of males and females, of those who

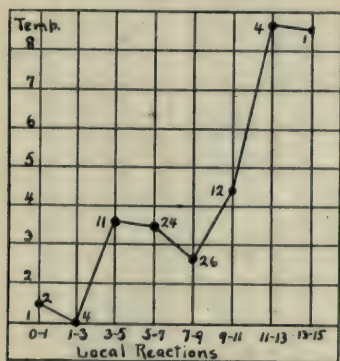


Fig. 2.—The relation between the severity of local reactions and temperature reactions following vaccination. The facts shown may be expressed thus: Among twenty-four subjects whose local reactions following the three inoculations averaged from 5 to 7 the average of highest recorded temperature following the three inoculations was 37.35 C.; among twenty-six subjects, etc.

had previously had typhoid fever or antityphoid vaccination and of those who had not, of nurses, of medical students, of dental and pharmaceutical students and of institutional help. The administration to all four groups was started at the same time, and the subjects were not told which vaccine they were receiving.

Thermometers were supplied and all were required to keep a temperature record, recording the readings as nearly as possible every three hours. Inasmuch as our experience has led us to suspect a considerable amount of suggestion in the reports of symptoms following vaccination, it was decided to use the temperature records alone as a basis for estimating the severity of the general reactions. In thus limiting the analysis it is of course recognized that the temperature is

but one symptom, and that undoubtedly certain subjects are made quite uncomfortable without a corresponding elevation of temperature. It is equally true, however, that the fever group includes the vast majority of those who suffer anything more than slight malaise or body aches, and that practically all those with no symptoms have no rise of temperature. The temperature records were found to agree with the subjects' valuation of their subjective symptoms when the results of the three inoculations were compared in these two ways (Figs. 4 and 5).

Likewise, in recording the severity of local reactions, the usual terms "slight," "moderate," and "severe" were replaced by definite measurements of the size of the local area of redness and of the

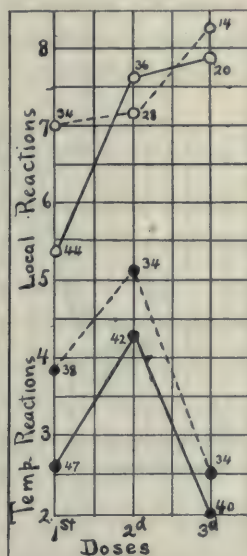


Fig. 3.—The relation of the vaccine used to the severity of reactions. Dots, circles and lines have the same significance as in Figure 1.

increase in circumference of the arm; even though here again it may not always be true that the amount of local discomfort is commensurate with the swelling and redness. At the time of administering the vaccine the circumference of the relaxed and loosely hanging arm was measured at the level of the needle puncture, and the next day this measurement was repeated. With an ordinary reaction the arm increases from 3 to 10 mm. in circumference. In order to obtain a single figure to represent the local reaction, this circumference increase in millimeters was averaged with the diameter of the red area measured in centimeters, according to the following equation:

$$\text{Local reaction} = \frac{1}{2} (10 \times \text{increase in arm circumference} + \sqrt{\text{longitudinal} \times \text{transverse diameter of red area}})$$

Before receiving the first dose of vaccine, each subject was given a typhoidin skin test, using the dry typhoidin powder according to the technic previously described.<sup>6</sup> Two weeks after beginning the vaccine administration the test was repeated; and again at three weeks, four weeks, five weeks, and from eight to ten months after the first dose of vaccine. The results of these tests were always read after about twenty-four hours and recorded not as merely "positive" or "negative" but as "typhoidin quotients,"<sup>6</sup> that is, the diameter of the typhoidin areola divided by that of the control. The technic was kept as uniform

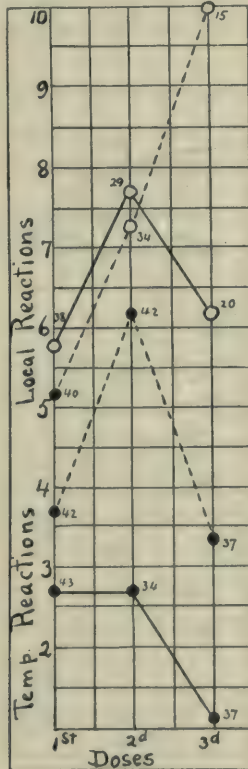


Fig. 4.—The relation of the interval between inoculations to the severity of the reactions. Dots and circles have the same significance as in Figure 1. Solid lines indicate ten-day schedule, broken lines two-day schedule.

as possible throughout the series of tests, and the same stock of typhoidin and control powders was used throughout. The measurements were made with a machinist's gage, and usually without knowing to which group the subject belonged.

1. *Relation Between Typhoidin Tests and Severity of Subsequent Vaccination Reactions.*—For showing this relation the record cards

6. Kilgore: THE ARCHIVES INT. MED., 1916, **17**, 25.



were arranged in the order of the typhoidin quotients at the beginning of the experiment, and on this basis separated into four classes with typhoidin quotients respectively 1.33 and under, 1.34 to 1.66, 1.67 to 1.99, and 2 and over. In each of these divisions those who received army vaccine were separated from those who received sensitized vaccine, and the average of the local reactions and of the general reactions determined, the reactions following all three doses being first averaged

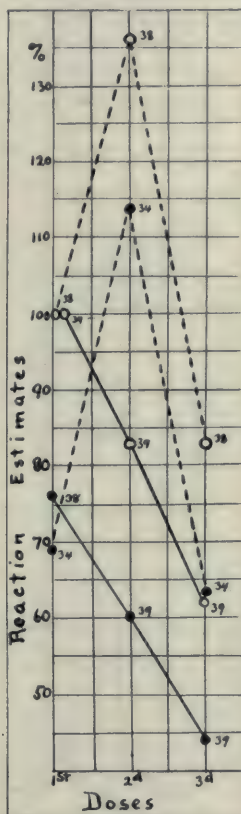


Fig. 5.—Same as Figure 4 except that the subject's estimates of their general and local reactions are used as the basis of comparison instead of temperature and arm measurements; the first reactions were taken by each subject as 100 per cent.

together for each subject. The results are shown in Figure 1, where the dots indicate temperature reactions, circles local reactions, solid lines those who received army vaccine, and broken lines those who received sensitized vaccine. In this figure, as well as those which follow, the numbers opposite the dots and circles indicate the number of persons on which the corresponding averages are based. It will be seen that the smallest local and general reactions occurred in those

with the lowest typhoidin quotients; that is, if the typhoidin quotient is regarded as a quantitative index of immunity against typhoid fever, the least discomfort from antityphoid vaccination was experienced by those most in need of protection. The severest local reactions occurred usually in those with highest typhoidin quotients. It will be noted, however, that the average of highest temperatures occurred in subjects with typhoidin quotients of midvalues, that is, from 1.34 to 1.66, and that no marked difference in this relation appears between the experience of those who took army vaccine and those who were given sensitized vaccine. Possibly in a larger series the highest average temperatures would be found associated with the largest preliminary typhoidin quotients.<sup>7</sup>

2. *Relation Between the Severity of the Local and Temperature Reactions Following Vaccination.*—On the other hand, we have no assurance that the local and constitutional reactions following vaccina-

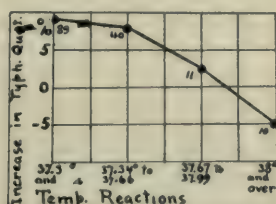


Fig. 6.—The relation between the severity of general reactions and the increase in typhoidin quotients during the next five weeks. Numbers at the left represent percentage gain in typhoidin quotients over the quotient obtained in the previous test; those at the bottom indicate the averages of the highest temperatures recorded after each of the three inoculations in each subject.

tion are due to the same allergic mechanism. The divergence of the curves representing general and local reactions in Figure 1 suggests a possible tendency for the severer local reactions to be more often associated with medium temperature. In order to test this association directly the subjects were divided into eight groups according to the average local reaction in each case following the vaccinations. As before, in each group the average temperature reaction was determined by averaging the highest temperatures recorded for each subject following each of the three doses of vaccine, and then taking the mean of all these averages. It will be seen from Figure 2 that the apparent

7. The general average of temperature reactions in the twenty-one subjects with preliminary typhoidin quotients of 1.67 or over is 0.42 C. According to the formula  $E. = \pm 0.6745 \frac{\sigma}{\sqrt{n}}$  (Davenport: *Statistical Methods with Special Reference to Biological Variation*, John Wiley & Sons, New York, 1914) the probable error of this average is  $\pm 0.09$  C. It is obviously impossible, therefore, to form any definite conclusions from the downward tendency toward the end of these curves.

tendency is for the highest temperatures to be associated with the severest local reactions and vice versa.<sup>8</sup> The apparent conflict between the facts represented by Figures 1 and 2 may easily be due to the paucity of observations.

3. *The Influence of Typhoid Fever or Vaccination on the Severity of Reactions Following Subsequent Antityphoid Vaccination.*—Not infrequently, on second vaccination, after an interval of one or two years the reaction is considerably more severe than at first; and the general impression has been that second vaccinations are likely to be much more disagreeable. Twenty-three subjects responded to a request to express roughly in figures the relative severity of their symptoms at this time and at the time of a previous vaccination. In nineteen out of this twenty-three the vaccination previously received was sensitized, and in only nine of them was the present administration of the same type. Some of these subjects had received their first vaccination when the method of preparing the sensitized vaccine made the dosage smaller than that now used. Analysis of these replies shows that if the pre-

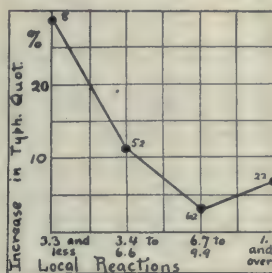


Fig. 7.—The relation between the severity of the local vaccination reactions and the gain in typhoidin quotient during the next five weeks. Same as Figure 5 except that the bottom numbers indicate grades of local reactions.

8. Since this compilation was made another series of sixty-three persons have been vaccinated. They belonged to the same class of subjects; forty-eight were male and fifteen female, and all were inoculated in the course of one week. In the order in which they appeared for the first dose, every other one was given sensitized vaccine (No. 805, manufacture completed four months previously) and every other one army vaccine (mostly No. 171, manufacture of which was completed from four to five weeks previously, a few of the second and third doses being from ampules several months old). The subjects were not told which vaccine they were being given, and the records of temperatures and local reactions were kept and analyzed as before. Since it was felt that the effect of the inoculation interval was fairly well established (see fifth section of this paper), and it was necessary to finish the work shortly, all were vaccinated according to the rapid schedule, every other day, except in thirteen persons, for whom it was more convenient to take the last dose after an interval of four days, that is, one week after the first dose. The figures from this second series substantiate Figure 2, since the average of highest temperatures of those with local reactions below 10 is 37.34 C.; of those with local reactions of 10 or over, 37.55 C.



vious experience is counted as 100 per cent., the general reactions at the present time averaged 197 per cent. and the local reactions 433 per cent. These figures express the general impression noted above, but they are necessarily very rough estimates, and it is conjectural how much the comparisons have been warped by the soothing influence of time and the freshness of the recent sting.

Also, from the relation described above of the reaction severity to the preceding typhoidin test, it would be expected that the less severe reactions would occur in those with negative history, especially since it has been shown<sup>9</sup> that the typhoidin test does bear a certain relation to the previous history of typhoid fever or vaccination. In the present experiments, however, the comparison of the average reactions of forty-eight subjects with negative history and of thirty-seven who had received antityphoid vaccination within the previous three years, or who had had typhoid fever, is as follows:

	Mean of Highest Tem- peratures, C.	Mean of Local Reaction
Negative history .....	37.33	7.3
History of typhoid or vaccination.....	37.36	6.5

The differences between these averages are too small for argument either way;<sup>9</sup> and while the series is not large enough to warrant final conclusions, it gives ground for strong suspicion that, other factors excluded, the supposedly greater average severity of symptoms following second vaccination is overrated, if it exists at all. After an interval of a year or two a number of subjects find a second immunization definitely less annoying.

4. *The Relation of the Vaccine Used to the Severity of Reactions.*—For this comparison the first two groups, which received army vaccine, were separated from the second two, which were given sensitized vaccine. Figure 3 (in which the symbols have the same meaning as in Figure 1) shows the relation of the local and general reactions following each of the three doses. Here again the results were contrary to our expectations, for in every instance except the local reactions following the second dose the higher averages for both local and temperature reactions occurred in the group which received sensitized vaccine. The differences, to be sure, are not very great, and in a larger

9. In the second series (Footnote 8) were forty-six with negative history and seventeen who had had typhoid fever or antityphoid vaccination within three years. The reaction differences between these two groups are as follows:

	Mean of Highest Temperatures, C.	Mean of Local Reaction
Negative history group.....	37.44	7.4
History of typhoid or vaccination.....	37.53	8.7

These differences, though slightly larger than in the first series and though they correspond with the prevailing notion of greater severity of second immunizations, are not large enough to modify essentially the opinions expressed above.

series it is possible that the relation would be reversed.<sup>10</sup> Obviously, differences in the strains from which the vaccines are derived may also affect the toxicity. But after this comparison involving eighty-five cases about equally divided (forty-seven receiving army vaccine, thirty-eight sensitized vaccine) it is not to be presumed that a larger series inoculated with the vaccines here used would show the decided difference we had expected to find in favor of the sensitized vaccine. The higher dosage of the army vaccine has also to be taken into account. With fresh preparations, therefore, the uncomfortable effects of the two vaccines are probably about equal.

10. In the second series (Footnote 8) the relation is reversed, thus:

	First Dose		Second Dose		Third Dose	
	Army V.	Sens. V.	Army V.	Sens. V.	Army V.	Sens. V.
Mean of highest temperatures....	37.33	37.32	37.86	37.52	37.39	37.38
Mean of local reactions.....	8.8	7.3	7.1	7	9.6	6.7

The differences, it will be seen, are small, and do not warrant change in the deductions stated after the first series. If the two series are combined the results are as follows:

	First Dose		Second Dose		Third Dose	
	Army V.	Sens. V.	Army V.	Sens. V.	Army V.	Sens. V.
Mean of highest temperatures....	37.45	37.51	37.81	37.61	37.56	37.53
Mean of local reactions.....	5	5.5	5.4	5.9	4.5	3.8

As a measure of the variability of the material and hence of the reliability or "precision" of the results given by the averages exhibited in Figure 3, the probable errors and "standard deviations" were worked out for the temperature averages following the first inoculation, using the formulæ  $E$  of  $M =$

$$\pm 0.6745 \frac{\sigma}{\sqrt{n}}; \text{ and } \sigma \text{ of } M = \sqrt{\frac{S}{n^2}} \text{ (Davenport: Footnote 7). The probable}$$

error of the average of highest temperatures following first inoculations of sensitized vaccine was found to be  $\pm 0.038 + C.$ , that is, the average of 37.39 C. for these thirty-eight subjects has about equal chances of being nearer or farther than 0.038 C. from the truth, as it would have been established by many thousands of observations. The corresponding probable error for the group of forty-seven who received army vaccine is 0.027 C. According to the formula  $A_1 - A_2 = \sqrt{E_1^2 + E_2^2}$  (Davenport: Footnote 7), the probable difference between the two averages is 0.047 C. It will be seen that these probable errors are not large in comparison with the separation of the two averages, a fact which creates a strong suspicion that the sensitized vaccine used at this time was really more pyrogenic than the army vaccine. A definite conclusion to this effect should not be made, however, without a consideration of the possible error. It is known that in frequency distributions of the symmetrical or moderately symmetrical type, such as these temperature averages might be expected to form if the work here reported were repeated many times on fresh material of the same kind, a range of six times the standard deviation constitutes the practical limits of variation, that is, it includes about 99 per cent. of all values of the variable (Yule, G.: *Introduction to the Theory of Statistics*, Charles Griffin & Co., London). The standard deviations of these averages are respectively 0.025 and 0.018 C. Six times the larger of these (0.025) is 0.15 C., which is a little more than the difference between the two averages. So that there is a possibility (though rather remote) that these two averages could have been derived from similar material; that is, that the showing of severer general reactions following the sensitized vaccine represents a simple "fluctuation of sampling" and not a real difference in the effects of the two vaccines.



5. *The Relation of the Interval Between Inoculations to the Severity of the Reactions.*—For showing this relation, Groups 1 and 3 (forty-two subjects), in which vaccine was administered every other day, are compared with Groups 2 and 4 (forty-three subjects), in which the dosage interval was ten days. The comparison is shown in Figure 4, where the dots indicate temperature reactions, the circles local reactions, solid lines the ten-day schedule, and broken lines the two-day schedule. It will be seen that the general reaction following the first dose was a little more severe in the two-day group than in the ten-day group, a difference not to be accounted for by the difference in dosage interval which followed. Allowance for this difference should be made, therefore, in comparing the results of the second and third inoculations in the two groups. After making this allowance, however, a substantial difference remains in favor of the ten-day schedule as the more comfortable.

The local reactions are approximately the same in the two groups for both the first and second inoculations, while following the third inoculation the two-day group, according to the measurements taken, had their severest reactions, and the ten-day group averaged considerably less. The practice was to vaccinate, about the insertion of the deltoid, the first time in the left arm, the second time in the right arm, and the third time in the left arm again. Fresh areas for inoculation were therefore used for the first and second doses, while for the third the first area was reinoculated. Thus in the two-day group the third injection was into an area which had been injected only four days previously; in the other group the interval was twenty days. This fact probably accounts for the difference in the local reactions following the third doses.

It is quite possible, however, that these measurements of the increase in arm circumference and size of the red area (especially the latter) in the arms reinoculated after a four-day interval do not accurately represent the relative discomfort to the subjects. In fact, the average estimate of the subjects themselves was not in agreement with this comparison of second and third local reactions. They were asked to state roughly in figures the relative severity of local and general symptoms following the three inoculations, taking the first as 100 per cent. The results are shown in Figure 5, where the dots, circles and lines have the same significance as in Figure 4. It will be seen that the estimates agree quite closely with the general reactions interpreted in terms of the highest temperatures, the temperature reaction following the second dose being specially high in the two-day group. The second local reaction was most severe according to the average testimony of the two-day group. In the ten-day group the averages for



both local and general reactions were thought to become less severe with each succeeding dose. The averages of the general reactions following the first dose are less than 100 per cent., because in some cases no general symptoms were noted at this time, and the reaction was, therefore, rated 0 per cent. In such cases, if any symptoms followed the second dose it was then called 100 per cent.

6. *The Influence of the Vaccine Used and of the Dosage Interval on the Extent and Rapidity of Immunity Production.*—It was hoped to obtain from the successive cutaneous typhoidin tests following vaccination some indication of which vaccine and which dosage interval produced immunity the more promptly or certainly. For the purposes of this comparison the typhoidin quotients obtained in these tests were expressed as percentage gains or losses, as compared with the preliminary typhoidin quotients. Thus, if the preliminary quotient was 1.5 and one of the later quotients 2, the result was put down as 33 per cent. gain, while if the later quotient was 1.25 there was a loss, which was recorded as —17 per cent. The algebraic sum of these plus and minus percentages was divided by their number to obtain the averages.

The numbers not being large enough (owing to the closing of the school year, many of the later tests were not obtained) to make profitable separate comparisons of the different weekly tests in each group, the results of second, third, fourth, and fifth weeks have all been averaged together and show the following comparisons:

In Group 1 (army vac., 2-day interval) gain in quot. (av. of 40 tests) = 13%  
In Group 2 (army vac., 10-day interval) gain in quot. (av. of 40 tests) = 3%  
In Group 3 (sens. vac., 2-day interval) gain in quot. (av. of 35 tests) = 5%  
In Group 4 (sens. vac., 10-day interval) gain in quot. (av. of 35 tests) = 11%

The average gain for all those who received vaccine every other day was 9 per cent., as compared with 7 per cent. for those on the ten-day schedule. The average for all those who received army vaccine was 8 per cent., and exactly the same for all those who received sensitized vaccine.

From eight to nine months after the vaccinations, as many as possible of these subjects were given another cutaneous typhoidin test, using the same stock of typhoidin and control powders. These typhoidin quotients were similarly compared with the prevaccination quotients, and the averages in each group are as follows:

In Group 1 (army vac., 2-day interval) gain in quot. (av. of 15 indiv.) = 1 %  
In Group 2 (army vac., 10-day interval) gain in quot. (av. of 11 indiv.) = 4.5 %  
In Group 3 (sens. vac., 2-day interval) gain in quot. (av. of 13 indiv.) = 20 %  
In Group 4 (sens. vac., 10-day interval) gain in quot. (av. of 12 indiv.) = 10 %

Again there is no marked difference between the two-day and ten-day groups, but those who received sensitized vaccine now show considerably greater average gain in typhoidin quotients than those who were given army vaccine.

This difference is not referable to differences in composition of the groups, for all contain about the same proportion of individuals who had received their first and only typhoid vaccination eight to ten months previously and those who had also had still earlier immunizations (the latter comprised 50 per cent. of the first two groups and 54 per cent. of the second two groups). Moreover, the greater gain in typhoidin quotients among those who received sensitized vaccine cannot be accounted for by a closer relation in this group between the immunizing vaccine and the typhoidin. The strain of organism used in preparing the typhoidin was the Dorset strain, while the army vaccine is prepared from the Remling strain, and the sensitized vaccine from strains isolated from four cases in California.

The table as it stands, therefore, suggests that sensitized vaccine is considerably more potent than army vaccine in producing well marked typhoidin skin tests eight to ten months after vaccination.

Before a definite conclusion to this effect can be drawn, however, it is necessary to consider the amount of variation in the figures from which these averages are derived and the strength of the probabilities that similar differences would be present if the groups were much larger. The high average gain of 20 per cent. in Group 3 would be reduced to 9 per cent. by the omission of one remarkably high quotient caused by a very wide but faint outer areola about the test spot.<sup>11</sup> The following table shows for each of the average typhoidin quotient gains its probable error and practical limits:<sup>12</sup>

	Mean Gain in Typh. Quot., %	Prob. Error of Mean, %	Practical Limits of Variation of Mean, %
Group 1.....	1	6	—18 to 36
Group 2.....	4.5	5	—17 to 25
Group 3.....	20	9	—20 to 60
Group 4.....	10	7	—21 to 41
Groups 1 and 2 (army vac.)..	2	4	—16 to 20
Groups 3 and 4 (sens. vac.)..	15	6	—10 to 40

It will be seen that by this consideration of the probable errors and practical limits, what at first appeared to be a rather striking and definite difference in favor of the sensitized vaccine is shown to be easily within the territory of experimental error. Evidently it would

11. Kilgore: Elsewhere in this number of this journal.

12. That is, a range of six times the standard deviation, Footnote 9.

be a mistake to base conclusions on the differences between these averages unless the differences were much greater or the series much larger or unless there were much more uniformity among the individual typhoidin quotient gains.

It is clear, therefore, that the cutaneous typhoidin test has revealed no superiority connected with the three-day or the ten-day inoculation interval, and in the first month after vaccination no difference between the two types of vaccine used. Eight to ten months later there is a probability (unfortunately quite a weak one) that those who have received sensitized vaccine will show the more marked cutaneous reactions to typhoidin. If the average typhoidin quotient of a group of individuals is assumed to be proportional to their resistance against typhoid fever, as was suggested by the finding of a higher average quotient among immunes than nonimmunes,<sup>6</sup> the observations here recorded may be looked on as favorable to the sensitized vaccine as the more efficient means of producing immunity. It must be emphasized, however, that as yet there is no definite warrant for such an assumption in regard to immunity, even if the higher typhoidin quotient following sensitized vaccine administration were borne out by adequate statistics.

*7. The Relation Between the Severity of the Reactions Following Vaccination and the Subsequent Gain in Typhoidin Quotients.*—By using the postvaccination typhoidin tests in the manner just described, the cases were separated into four classes according to the severity of the temperature reactions: those whose highest temperatures following the three vaccinations averaged (1) 37.33 C. or less, (2) from 37.34 to 37.66 C., (3) from 37.67 to 37.99 C., and (4) 38 C. or over, and the average increase in typhoidin quotients was calculated for each class. The results are shown in Figure 6, where the numbers at the bottom indicate the four grades of temperature reactions and those at the side the percentage gain in typhoidin quotients during the following five weeks. The results of the typhoidin tests from eight to ten months after vaccination are not shown, since the observations were so few that the averages were markedly discordant.

In Figure 7 the same sort of comparison is shown with the cases divided according to the severity of the local reactions, the numbers at the bottom indicating grades of local reaction obtained according to the formula already given.

Figures 6 and 7 suggest that those who have the highest temperatures and the greatest amount of arm swelling and redness from the vaccination are the ones who show the least increase in their typhoidin quotients. Or, conversely, that if it is true that the typhoidin test



applied to groups gives a quantitative index of typhoid immunity, those who suffer least discomfort from the vaccination derive the greatest benefit from it. This of course is the result to be anticipated from observations already made. For the group which has the best chances of showing the greatest average gain in typhoidin quotient is obviously the group with the low preliminary quotients; and these are also (Fig. 1) the ones with the lesser reactions following vaccination.

The numbers are not large enough to make profitable a separate comparison for the different vaccines or methods of administration.

#### CONCLUSIONS

Final conclusions are not warranted from the number of observations in this series, especially since in some particulars they would be at variance not only with what seemed to be my own experience, but also with that of Gay, Force, and others. Since, however, some changes have been made in the dosage and preparation of the sensitized vaccine since our former impressions were received, and since more than the usual precautions were taken at this time to eliminate the personal equation in the results, the following findings may be put forth with the understanding that they are to be given weight only in accordance with the number of observations on which they are based:

1. The higher the typhoidin quotient in a given person, the greater his chances of a severe reaction to antityphoid inoculation.
2. The severer local reactions to vaccine tend to occur in those subjects with the higher temperature reactions.
3. The supposedly more severe average reaction to antityphoid inoculation of subjects who have previously had the treatment is probably overrated.
4. The use of army vaccine or sensitized vaccine sediment if the preparations are fresh makes little if any difference in the average local and temperature reactions.
5. When either type of vaccine is administered every other day the temperature reactions following the second and third doses are likely to be more severe than when the injections are ten days apart. A difference in the local reactions is not so clear cut. The average impression of the subjects vaccinated every other day is that the second injection gave the greatest local and general discomfort and the third least. The average member of the ten-day group feels that both general and local reactions were less with each succeeding dose.
6. The typhoidin test failed to show any noteworthy differences in effectiveness between the two-day or ten-day schedules, and, in the

first few weeks, between the two vaccines used. Later the typhoidin quotients were higher in those who had received sensitized vaccine, but the differences are not decided enough to support definite conclusions that this is the rule.

7. According to the typhoidin test it is suggested that the ones who react least to vaccination tend to profit most by its administration.

# THE AGGLUTININS AND COMPLEMENT-FIXING ANTIBODIES IN THE SERUM OF PERSONS VACCINATED AGAINST TYPHOID FEVER \*

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During the last few years numerous reports have appeared on the value of the Widal reaction in vaccinated persons. Most of these studies suffer from lack of uniformity of technic or from faulty conclusions derived from a study of selected cases only. In the European armies the extensive use of typhoid vaccines of varying antigenic value and different modes of preparation has especially called forth numerous publications on the value of serologic tests as a means of ascertaining the grade of immunity present. Unfortunately, the results are not comparable on account of the diversity of the preparations and of the methods used in the different countries. Most of the writers consider only the agglutination test (their results will be discussed in connection with ours). Felke<sup>1</sup> and also Hage and Korff-Petersen,<sup>2</sup> however, report as well on the value of the complement fixation test; thus Felke claims to be able to distinguish patients who had had typhoid fever from those who had been vaccinated, by the absence of complement fixing antibodies in the serum of the latter. Nine months after the last vaccination, however, Hage and Korff-Petersen found in a few of the persons decided fixation of the complement.

Wollstein<sup>3</sup> and Howell,<sup>4</sup> in this country, studied carefully the various immune bodies and in four patients and two patients, respectively, vaccinated with Russell's preparation, according to the United States Army method. Wade and McDaniel,<sup>5</sup> Moon,<sup>6</sup> Hamilton,<sup>7</sup> Rüdiger

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1. Felke: Die Komplementablenkung als Reaktion zur Unterscheidung zwischen den Seren Typhuskranker und gegen Typhusgeimpfter, München. med. Wchnschr., 1915, **62**, 578.

2. Hage and Korff-Petersen: Typhusschutzimpfung und Typhusdiagnose, Deutsch. med. Wchnschr., 1915, **45**, 1328.

3. Wollstein, M.: The Duration of the Immune Bodies in the Blood after Antityphoid Inoculation, Jour. Exper. Med., 1916, **16**, 315.

4. Howell, Katherine: Observations on the Production of Antibodies after Antityphoid Inoculation, Jour. Infect. Dis., 1916, **19**, 63.

5. Wade, E. M., and McDaniel, O.: Observations on the Widal Reaction Following the Administration of Typhoid Vaccine, Am. Jour. Pub. Health, 1915, **5**, 136.

6. Moon, V. H.: Experimental Immunity in Relation to the Agglutination Reaction in Typhoid, Jour. Am. Med. Assn., 1913, **60**, 1764.

7. Hamilton, C. D.: The Effect of Typhoid Vaccination on the Widal Reaction, Jour. Am. Med. Assn., 1915, **65**, 1873.



and Hulbert,<sup>8</sup> and others tested the Widal reaction in those vaccinated with preparations of their own make. In these instances the microscopic agglutination test was employed and no definite quantitative determinations were attempted.

Thus far no data are available concerning the serum reactions in human beings vaccinated with the sensitized, detoxicated vaccine sediment of Gay and Claypole.<sup>9</sup> From experimental work done on rabbits by Nègre<sup>10</sup> and Löwy<sup>11</sup> with living sensitized bacilli, and by Gay and his co-workers with the killed sensitized typhoid bacilli, it has become known that animals so vaccinated develop agglutinins only in moderate concentrations as compared with those in which the untreated typhoid antigen is used. On the other hand, according to Nègre and Löwy, the serums of animals immunized with sensitized vaccines contain the complement-fixing antibodies and the bactericidal substances in larger, or at least in the same, amounts as compared with those treated with non-sensitized vaccines.

The growing importance of typhoid immunization, the continued uncertainty in regard to the best type of vaccine, optimum interval between doses, and the interval after which revaccination becomes advisable, as well as the desire of physicians and laity for a practical test of typhoid immunity, make important any work which may throw light on this subject. In view, therefore, of the unsettled state of the literature concerning typhoid serologic changes, it was considered important, in connection with a new series of cutaneous typhoidin tests (reported by one of us elsewhere in this number of this journal) to investigate in the same subjects at the same time the phenomena of agglutination and complement fixation.

The subjects consisted of ninety-eight students, nurses and others connected with the University of California Departments of Medicine, Dentistry and Pharmacology; most of them were young adults and all in apparently good health. Seventeen had had typhoid fever from three and one-half to twenty-five years ago (in most cases not proved by biologic methods); twenty-six had received army vaccine<sup>12</sup> from

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8. Rüdiger, G. F., and Hulbert, Robert: Is Blood as Reliable as Fresh Serum in Making the Widal Test? *Am. Jour. Pub. Health*, 1914, **4**, 113.

9. Gay, Frederick P., and Claypole, Edith J.: An Experimental Study of Methods of Prophylactic Immunization Against Typhoid Fever. *Studies in Typhoid Immunization*, V, *THE ARCHIVES INT. MED.*, 1914, **14**, 671.

10. Nègre, L.: Recherches comparatives sur la disparation des reactions humores des lapins immunisés avec des bacilles typhique vivantes sensibilisés, tués par la chaleur et tués par l'éther, *Compt. rend. Soc. de biol.*, 1913, **75**, 412; *ibid.*, **74**, 1177.

11. Löwy, O.: Immunkörperbildung verschiedenartiger Typhusimpfstoffe, *Deutsch. med. Wchnschr.*, 1915, **45**, 1277.

12. The army vaccine was obtained through the courtesy of Capt. Henry J. Nichols of the U. S. Army Medical Corps. The details relative to these vaccinations are given by one of us elsewhere in this number of this journal.

three to eight months previously, and thirty-seven had been given sensitized vaccine<sup>13</sup> within the same time. The term "vaccination" as here used implies in all cases the full three inoculations. In the use of both types of vaccines about one-half the persons had been inoculated at intervals of three days and the others at ten-day intervals. These dosage intervals are not specified in the tables herewith, for, as Cahn-Bronner<sup>14</sup> has recently shown, irregularity of vaccination has no influence on the formation of antibodies.

#### AGGLUTINATION TESTS

*Method.*—The use of a uniform, standardized antigen for comparative tests is of the greatest importance. Dreyer and Walker<sup>15</sup> and co-workers have clearly pointed out the fallacies of the old microscopic method and the unreliability of the agar suspensions, and for years it has been our practice to use broth cultures in tests with organisms of the paratyphoid group.<sup>16</sup> Liebig's broth cultures of the typhoid bacillus were treated either with phenol (0.5 per cent.) or with formaldehyd solution (0.1 per cent.) and carefully standardized with rabbit immune serums. Liebig's broth, obtained from one and the same lot of preparations and having a reaction of 0.85 per cent. ( $p_H^+$  7.2) was used. All the strains of *Bacillus typhosus* had been previously subcultivated in Liebig's broth daily for two weeks. The typhoid strains employed included the army strain (Rawlings), and four others (M., L., B., and Sch.), which were known to be present in the sensitized vaccine sediment. Separate series for each strain were set up and the average agglutination titer was calculated from the five agglutination reactions of each serum. This average is given in the tabulated results.

Fresh and perfectly clear serum from proper dilutions in amounts varying from 0.1 to 0.01 c.c. was introduced with capillary pipets into narrow, suspended tubes, and to each 1 c.c. of the standardized antigen was added. After carefully shaking, the tubes were incubated for two hours at 37 C., and the results noted. Final readings, however, were made only after an interval of twelve hours. In the tables is recorded only complete agglutination in twelve hours with a perfectly clear, supernatant fluid.

13. The regular product supplied during the last three years by the California State Hygienic Laboratory.

14. Cahn-Bronner, C. E.: Typhus Schutzimpfung und Typhusdiagnose bei Geimpften, Med. Klin., 1915, **11**, 964.

15. Dreyer, Georges: Widal's Reaction with Sterilized Cultures, Jour. Path. and Bacteriol., 1909, **13**, 332. Walker, E. W. Ainley: A Note on Widal's Reaction with Standardized Agglutinable Cultures, Lancet, London, 1916, **1**, 17.

16. Meyer, K. F., and Boerner, F.: Studies on the Etiology of Epizootic Abortion in Mares, Jour. Med. Research, 1913, **29**, 325.

Table 1 contains a summary of the results obtained in ninety-two cases, which are divided into five large groups, according to their histories. The first group consists of twenty-three persons who had been vaccinated with army vaccine from three to eight months previously. Of these, twelve had then been vaccinated for the first time, while eleven had also had a previous course of immunization with either sensitized or army vaccine.

TABLE 1.—PERCENTAGE OF AGGLUTINATION REACTIONS

Group	Vaccine Preparation	Time Interval	Dilutions of Serum						No. Cases
			1:40	1:60	1:80	1:100	1:200	1:400	
1	Army vaccine, first time...	8 mo.	100	80	60	50	50	10	10
	Army vaccine, first time...	3 mo.	100	100	100	50	50	.....	2
	Army vaccine, repeated inoculations	8 mo.	90.9	55.5	36.3	27.2	9	9	11
2	Gay-Claypole sensitized vaccine sediment, first time	7 to 10 mo.	57.1	21.4	14.2	14.2	.....	.....	14
	Gay-Claypole sensitized vaccine sediment, first time	2 mo.	33.3	.....	.....	.....	.....	.....	3
	Army vaccine, repeated inoculations	8 mo.	70.5	29.4	23.5	5.8	.....	.....	17
	Army vaccine, repeated inoculations	6 mo.	100	.....	.....	.....	.....	.....	1
		2 mo.	100	100	100	.....	.....	.....	1
3	Negative history.....	.....	52.9	11.7	11.7	.....	.....	.....	16
4	Typhoid fever.....	3½ to 25 yr.	60	30	20	.....	.....	.....	10
5	Typhoid fever and vaccinated with army vaccine	6 to 8 mo.	60	40	20	20	20	.....	5
	Typhoid fever and vaccinated with Gay-Claypole sensitized vaccine sediment	6 to 8 mo.	50	50	50	50	.....	.....	2

In the columns under the various serum dilutions are recorded the percentage of cases giving complete agglutination. It will be seen that this group, as a whole, shows a well-marked persistence of agglutinins, regarded either from the standpoint of agglutination in high dilutions or high percentage of individuals having a moderate amount of agglutinins. The slightly greater percentage of high dilution agglutinations among those who had had but one course of immunization depends on too few observations to merit an attempt to interpret it.

The second group consists of thirty-six persons who had been



vaccinated with Gay-Claypole<sup>9</sup> sensitized vaccine sediment from two to ten months previous to the test. For thirteen of them this vaccination was the first and only typhoid immunization; the other nineteen had been immunized one or more times before with sensitized or army vaccine.

This entire group shows a very low agglutination titer. Thus, only 14.2 per cent. agglutinate *B. typhosus* in a dilution of 1 to 100. Over 40 per cent. failed to agglutinate in a dilution of 1 to 40 and, from the records not tabulated, over 23 per cent. of the serums failed to agglutinate the typhoid bacillus even in a dilution of 1 to 20. In this respect the group shows analogies to Group 3, in which neither the history of typhoid fever nor previous typhoid inoculation was obtainable. Some irregular cases may show agglutination in higher dilutions; but, as will presently be shown, the same may also happen in the "negative history" group.

The third group comprises sixteen persons who gave no history of antityphoid vaccination or typhoid fever. With our standardized typhoid antigen the serum of such an individual will rarely agglutinate in a dilution higher than 1 to 40; and this statement conforms well with the average of thousands of tests reported in the literature.<sup>17</sup> It is well known that the agglutinability of the strains employed for such tests will influence the results. Our strains, no doubt, were easily agglutinated. The average of nearly 50 per cent. of the serums gave an agglutination in a dilution of less than 1 to 40 and about 10 per cent. failed to agglutinate the typhoid bacillus. One case, with a negative history, gave an agglutination of 1 to 80 and a marked complement fixation, together with a very high typhoidin quotient (2.29). In view of the undoubtedly large number of undiscovered cases of typhoid fever,<sup>18</sup> it is not improbable that if the facts were known, this case would be classed in Group 4 with the typhoid recoveries.

The fourth group includes ten subjects who gave a definite history of having had typhoid fever from three and one-half to twenty-five years before. The serums of this group behaved, in many respects, like those of Group 3. Thus, the percentage of agglutination is low, only about 40 per cent. of the serums giving negative reactions, or agglutinating in dilutions below 1 to 40. In only two cases was a positive result noted in a dilution of 1 to 80; both of these subjects had typhoid fever from ten to twelve years previously. In some persons therefore, the agglutination titer apparently persists for a long period

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17. Fornet, W.: Immunität bei Typhus, Kolle-Wassermann Handbuch d. pathogenen Mikroorganismen, Ed. 2, G. Fischer, Jena, 1914, **3**, 845.

18. Sawyer, Wilbur A.: Ninety-Three Persons Infected by a Typhoid Carrier at a Public Dinner, Jour. Am. Med. Assn., 1914, **63**, 1537.

after an attack of typhoid fever. Donges<sup>19</sup> and others made the same observation, but failed to find that this condition is influenced by another intercurrent disease. But according to the experimental studies of von Wassermann and Sommerfeld<sup>20</sup> it is not unlikely that an increase in the agglutinins may occur during the development of an intercurrent infection. No such cause was present, however, in our two cases.

In the fifth group are five persons who had had typhoid fever and who subsequently also had been vaccinated with sensitized vaccine sediment or army vaccine. Some of these had received as many as three courses of inoculations, the last vaccination in all cases being from six to eight months before our tests. From these few observations there is after a few months little apparent tendency of the vaccination to modify the low agglutinin concentration of the typhoid recoveries.

From experimental work on man (Moon<sup>21</sup>) and animals (Cole<sup>22</sup> and others) we may infer that in subjects who have had typhoid fever or antityphoid vaccination a subsequent injection of typhoid vaccine calls forth immune bodies rapidly and in considerable amounts; but that these immune bodies are also quick to disappear. Evidently, in all but one case their subsidence had taken place during the six or eight months between the last vaccination and our tests.

In the chart are shown graphically our most striking serologic findings, that is, the differences between Groups 1 and 2, those who had had army vaccine and those who had received sensitized vaccine. From the results after from three to eight months, it will be seen that the army vaccine appears to have considerably greater influence than sensitized vaccine in promoting the persistence of agglutinins in high concentration. This great potency of the army type of vaccine in stimulating the development of agglutinins has been previously shown by Russell<sup>23</sup> and by Wollstein.<sup>3</sup>

Dissimilarity in technic and in the preparation and dosage of the various vaccines forbid a strict comparison of our results with those of the British and German writers. The army vaccine, however, being a preparation of typhoid bacilli killed by heat, is similar in composition

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19. Donges: Ueber die agglutinatorische Kraft des Serums nach überstandener Typhusinfektion, *Centralbl. f. Bakteriol.*, 1914, **75**, 174.

20. V. Wassermann and Sommerfeld: Experimentelle Untersuchung über Typhus Schutzimpfung, *Med. Klin.*, 1915, **11**, 1307.

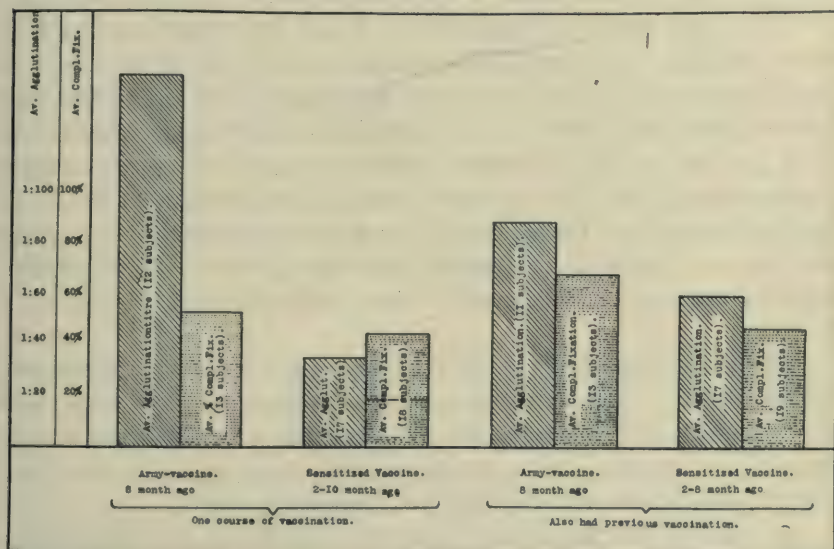
21. Moon, V. H.: Observations on Antibody Formation in Typhoid, *Jour. Infect. Dis.*, 1914, **14**, 56.

22. Cole, R. I.: Experimenteller Beitrag zur Typhusimmunität, *Ztschr. f. Hyg. u. Infektionskrankh.*, 1914, **46**, 371.

23. Russell, F. F.: Antityphoid Vaccination. The Immediate Results of the Administration of 3,600 Doses, *Bull. Johns Hopkins Hosp.*, 1910, **21**, 83.

and antigenic effect to the Wright or Pfeiffer-Kolle vaccine; so that many of the reports concerning agglutinins in vaccinated soldiers may be used for comparison.

In general, our figures, for those who had received army vaccine, are lower than those of the English writers and higher than those of German writers. Stieve,<sup>24</sup> for instance, states that the maximum agglutination which he obtained with serums from soldiers eight to twenty-one days after the last inoculation with Russell's vaccine was 1 to 600. However, we have observations on hand which show that the titer of 1 to 4,000 or 1 to 6,000, obtained with broth cultures treated with formaldehyd, six days after the second inoculation are not at all exceptional.<sup>4</sup>



Graphic showing of agglutination and complement fixation averages with army vaccine and sensitized vaccine.

Dreyer and Inman<sup>25</sup> emphasize the persistence of agglutinins in the blood of vaccinated soldiers and state that they exist for a longer period in the blood than is usually thought to be the case. In persons inoculated with two or more doses of vaccine a higher agglutination titer was noted than in those who received only one dose. Dakeyne<sup>26</sup> came to the same conclusions: he states that a gradual diminution in

24. Stieve, H.: Beobachtungen bei der Typhusschutzimpfung mit dem Russell'schen Impfstoff, München. med. Wchnschr., 1915, **62**, 237.

25. Dreyer, G., and Inman, A. C.: Persistence of Antibodies in the Blood of Inoculated Persons as Estimated by Agglutination Tests, Lancet, London, 1915, **2**, 225.

26. Dakeyne, D. I.: Observations on Some of the Agglutination Reactions of the Blood of Soldiers Inoculated Against Typhoid Fever, Lancet, London, 1915, **2**, 540.



the agglutination reaction results at the end of the eighth month. Dyer<sup>27</sup> found that five months after vaccination fifteen persons out of twenty-eight gave an agglutination in a dilution of 1 to 80, five in 1 to 60, and one in 1 to 320; and not in one instance was an agglutination less than 1 to 40 obtained. Cahn-Bronner<sup>14</sup> made the observation that in a large number of tests with Ficker's typhus diagnostikum, over 50 per cent. of German soldiers still gave an agglutination in a dilution of 1 to 50 after 240 days, and that over 38.5 per cent. reacted in a dilution of 1 to 100, and 23 per cent. in 1 to 200. Klose,<sup>28</sup> Klemperer, Oettinger and Rosenthal,<sup>29</sup> Weichhardt,<sup>30</sup> Ziersch,<sup>31</sup> Dünner,<sup>32</sup> Reiss,<sup>33</sup> and Hage and Korff-Petersen<sup>2</sup> noted the persistence of immune substances in soldiers vaccinated with different preparations.

Wade and McDaniel<sup>5</sup> state, however, that the Widal reaction is of short duration following the administration of typhoid vaccine. In only 16.3 per cent. of the cases was it still present after six months, and after one year only 11.7 per cent. gave a microscopic agglutination in a dilution of 1 to 50. Hamilton,<sup>7</sup> using an equally unreliable antigen, reports that all the vaccinated persons gave an agglutination in 1 to 50 up to the twenty-fourth month following the last injection. According to Bloch and Creuzé,<sup>34</sup> one half of the subjects vaccinated with Chantemesse preparation still gave one year later an agglutination of 1 to 100.

Our results, indicating as they do the large effect on the persistence of agglutinins which may come from variations in the method of preparing the vaccine, constitute an instructive commentary on the conflicting literature, and strongly suggest the need for better controlled experiments in attacking this complex problem.

In view of the considerable amount of variation in agglutination

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27. Dyer, A. W.: The Agglutination Reaction after Antityphoid Inoculation, *Indian Jour. Med. Research*, 1913-1914, **1**, 728.

28. Klose: Die Gruber-Widal'sche Reaktion bei typhusschutzgeimpften Franzosen und ihre Bewertung für die Diagnosestellung, *Arch. f. Hyg.*, 1915, **74**, 193.

29. Klemperer, F., Oettinger, W., and Rosenthal, F.: Zur Diagnostik und Therapie des Typhus im Felde, *Therap. d. Gegenw.*, 1915, **17**, 162.

30. Weichhardt, W.: Ueber Typhusimmunisierung, *München. med. Wchnschr.*, 1915, **62**, 431.

31. Ziersch, P.: Beobachtungen bei Typhusschutzgeimpften, *München. med. Wchnschr.*, 1915, **62**, 1312.

32. Dünner, L.: Die Bedeutung der Widal'schen Reaktion bei Typhusgeimpften Soldaten, *Berl. klin. Wchnschr.*, 1915, **52**, 59, 683.

33. Reiss: Der Wert der Agglutinations Probe bei Typhusgeimpften, *München. med. Wchnschr.*, 1915, **62**, 1277.

34. Bloch, M., and Creuzé, Pierre: Reactions humorales consecutives a l'emploi du vaccin antityphoide de Chantemesse, *Compt. rend Soc. de biol.*, 1912, **73**, 566.

titer among individuals of the same group and the limited number of observations, it is not possible from our results to make any statement in regard to the rate with which agglutinins disappear from the circulation of vaccinated persons. Klemperer, Oettinger, and Rosenthal<sup>29</sup> report irregularity in the rate of agglutinin subsidence in contrast to the regular, gradual decrease which is noted in cholera vaccination. Cahn-Bronner reported that in different cases the titer declined slowly or rapidly after the eighty-fifth day following the last injection, but that the change was always regular. For example, according to numerous observations, if a case showed a high agglutination four months after the initial vaccination, the decrease in the following months would be slow. On the other hand, in a case with a very low titer, the change to a negative agglutination within the same time interval would be more precipitous. This may be the explanation of the low values obtained in some of our cases of Groups 1 and 2.

Mention should here be made of the possible relationship between the intensity of the reaction following the administration of the vaccine and the subsequent agglutination titer. The early conception of Hetsch and Kutscher<sup>35</sup> that the agglutination titer is influenced by the clinical reaction has again been supported by Dakeyne. On the contrary, Cahn-Bronner, Klose and others fail to find any such relationship.

In our series one subject, on account of the severity of the reaction, was inoculated with only two doses of army vaccine, and her agglutination titer was still 1 to 400 eight months after the last injection. Three other cases, however, with similarly severe reactions, showed a low titer. From these observations it is apparent that to what extent vaccination reactions influence the degree of antibody production cannot be stated with any certainty from the data at hand.

Finally, in the light of the irregular persistence of the agglutinins in the serum of vaccinated persons, the Widal test in its usual clinical form has lost its diagnostic value in these cases. When, however, repeated quantitative determinations are made with the serum of the suspected typhoid patients, a change in the fluctuations of the titer may be of diagnostic significance, as has been shown by Dreyer and his pupils<sup>36</sup> in a large number of cases.

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35. Gaffky, Kolle, Hetsch, and Kutscher: Ueber Typhusschutzimpfungen, *Klin. Jahrbücher*, 1905, **14**, 124.

36. Davison, Wilburt C.: The Diagnosis of Enteric Fever (Typhoid and Paratyphoid A and B) by Agglutination Tests, *Jour. Am. Med. Assn.*, 1916, **46**, 1297. Klieneberger: Agglutinationstiter bei Infektionskrankheiten, *ims besondere Typhus und Paratyphus*, *Deutsch. med. Wchnschr.*, 1914, **40**, 1511.



## COMPLEMENT FIXATION TESTS

Our method was a modification of the ordinary Wassermann technic, the system being based on the use of a total fluid bulk of 2.5 c.c. of a 1 per cent. blood cell suspension. Because of the reliability of the technic a few details in connection with it may deserve mention.

Lemco<sup>37</sup> broth cultures about eighteen to twenty hours old were used as antigen. Younger cultures are frequently not sufficiently antigenic, and older ones are very anticomplementary. The advantage of these broth cultures over suspensions of extracts has been demonstrated repeatedly by Bordet, Massol and Grysez<sup>38</sup> and others.

The broth cultures were heated for one-half hour at 80 C., and preserved with 1 per cent. glycerin and 0.5 per cent. phenol (carbolic acid). Kept cool and protected from the light, they remained unchanged for the entire month during which they were employed for these tests. Four antigens were used: (1) broth culture of the army strain (Rawlings); (2) broth culture of thirty different strains of *B. typhosus*,<sup>39</sup> (3) broth culture of a strain of *B. coli* isolated from a case of cystitis; and (4) a polyvalent aqueous extract of thirty strains of *B. typhosus* prepared according to Dean.<sup>40</sup> This antigen was used only in some control tests.

The dose of the antigen chosen was four times the antigenic unit as determined by preliminary titrations of the antigen with a standard amount of rabbit immune serum, either 0.1 c.c. or 0.2 c.c. This dose was at least from one fourth to one fifth of the anticomplementary unit, as determined in the preliminary test. The range of the specific antigenic properties of each antigen was further controlled by numerous tests with serums of known antibody content.

The serum was separated from the blood clot by centrifugalization and kept in small vials at refrigerator temperature. Shortly before use they were inactivated at 56 C. for one-half hour. A standard amount of 0.1 c.c. was titrated against decreasing amounts of the antigens, the largest amount being four times the antigenic unit. Furthermore, in about 50 per cent. of the tests decreasing amounts of serum were titrated against a standard amount of antigen. Ordinarily, three such titrations were carried out in the above-mentioned broth cultures.

The antishoop hemolytic serum was used. Complement was fur-

37. Lemco, Method of preparation in, Eyre, T. W. H.: The Elements of Bacteriological Technique, Ed. 2, 1913, 163.

38. Massol, L., and Grysez, V.: Antigènes et anticorps typhiques. Reaction d'inhibition, Compt. rend. Soc. de biol., 1913, **75**, 220.

39. The senior author is indebted to Dr. F. P. Gay for some of the strains of *B. typhosus* used in this antigen.

40. Dean, H. R.: Studies on Complement Fixation with Strains of Typhoid, Paratyphoid and Allied Organisms, Ztschr. f. Immunitätsforsch. u. exper. Therap., 1911, **11**, 58.



nished by the pooled serums of at least two guinea-pigs, and was employed in a dosage of 0.05 c.c. of a dilution of 1 to 4 in salt solution. The red corpuscles were used in a 1 per cent. suspension.

A preliminary test was made of the hemolytic system, using 0.05 c.c. of complement and 0.5 c.c. of a 1 per cent. suspension of fresh sheep cells. In the titration of the antigen, as well as for the actual complement fixation tests, two hemolytic units (about 0.2 to 0.1 c.c. of a dilution of 1 to 100 in saline) were employed.

Preliminary tests were made to rule out any anticomplementary activity of the different antigens used. These were carried out as follows:

To each series of tubes containing decreasing doses (from 0.8 to 0.7 c.c.) of antigen diluted with 1.5 c.c. of salt solution was added 0.05 c.c. of complement in a dilution of 1 to 4. The tubes were incubated at 37 C. in a water-bath. To each tube was then added a previously prepared mixture of two units of hemolysin and 0.5 c.c. of the corpuscle suspension. After mixing and incubating for one hour, sedimentation of the red cells was hastened by placing the tubes in the refrigerator so as to make the readings more specific. In using a 1 per cent. suspension, very clear results are obtained, which make possible a quantitative expression of the degrees of fixation.

The actual tests with the three antigens were carried out in the following manner: To each 0.1 c.c. of serum was added 0.05 c.c. of complement in 1.5 c.c. of salt solution, followed by decreasing amounts of antigen. After incubation for a period of one-half hour the sensitized corpuscle suspension was added to each tube in the dosage already given. The mixture was then re-incubated for from one to two hours and the readings made already indicated.

The customary controls for the serums, antigen, hemolytic system were employed in duplicate at the beginning and at the end of each series.

On previous occasions we made the observation that human serums may show, in a few instances, nonspecific fixation in dilutions of 0.2 c.c. with broth antigens. To rule out such nonspecific reactions we made use of *B. coli* antigen, and all the serums which gave fixation with this antigen, even when 50 per cent. hemolysis was noted, are regarded as nonspecific. Several serums of women were anticomplementary in double or single amounts (from 0.2 c.c. to 0.1 c.c.); these are mentioned, therefore, in a separate column. The range of specific fixation is considerable with broth antigens in comparison with extracts or suspensions and is, in part, responsible for the number of reactions recorded. In doubtful cases we tested the serums also with extract antigens, and the results confirmed those noted with our regular preparations.

The three complement fixation tests on ninety-eight persons are summarized in Table 2. These include the subjects tested for agglutinins, and also six others from whom insufficient blood was obtained for both experiments. The cases are again divided, according to their previous history, into five main groups.

TABLE 2.—PERCENTAGE OF COMPLEMENT FIXATION REACTIONS

Group	Vaccine Preparation	Time Interval	Amount of Antigen Fixed by 0.1 C.c. Serum				Anticomplementary or Autotropic Serum	No. Cases
			0.2	0.1	0.05	0.02		
1	{ Army vaccine, first time....	8 mo.	45.4	27.2	27.2	.....	.....	11
	{ Army vaccine, first time....	3 mo.	100	100	50	50	.....	2
	{ Army vaccine, repeated inoculations	8 mo.	69.2	80.7	15.3	.....	(15.3)	18
2	{ Gay - Claypole sensitized vaccine sediment, first time	7 to 10 mo.	13.3	13.3	.....	.....	(20 )	15
	{ Gay - Claypole sensitized vaccine sediment, first time	2 mo.	66.6	.....	.....	.....	(33.3)	8
	{ Gay - Claypole sensitized vaccine sediment, repeated inoculations	8 mo.	52.9	23.5	17.6	.....	.....	17
	{ Gay - Claypole sensitized vaccine sediment, repeated inoculations	6 mo.	.....	.....	.....	.....	.....	1
	{ Gay - Claypole sensitized vaccine sediment, repeated inoculations	2 mo.	100	100	100	.....	.....	1
3	Negative history.....	.....	5.5	.....	.....	.....	(16.6)	18
4	Typhoid fever.....	3½ to 25 yr.	10	10	.....	.....	(10 )	10
5	{ Typhoid fever and vaccinated with army vaccine	6 to 8 mo.	80	40	40	.....	.....	5
	{ Typhoid fever and vaccinated with Gay-Claypole vaccine	6 to 8 mo.	50	50	.....	.....	.....	2

The first group comprises twenty-six persons who had been vaccinated with army vaccine three to eight months previous to the tests. Thirteen of these were immunized for the first time, and the same number had had repeated inoculations with either army or sensitized vaccine. In this group a fairly high percentage, about 45.5 per cent., furnished serums which even in amounts of 0.1 c.c. fixed the specific antigen in quantities of 0.2 c.c. Detailed records of the test indicate that the army strain gives somewhat better fixation than the polyvalent

antigen; and, in the light of the recent findings of Hooker,<sup>41</sup> the discussion of Garbat<sup>42</sup> that the *B. typhosus* strain "Rawlings" differs immunologically from many recently isolated strains of typhoid organisms, this observation is readily explained. Only about 27.2 per cent. of the serums contained large amounts of fixing antibodies, most of them giving reactions only with the largest amount of antigen used in the tests. In two serums derived from individuals who had been vaccinated three months previous to the test the complement fixing antibodies were still high, and in one sample even higher than the agglutinins (1 to 80). It is quite apparent that the fixing antibodies disappear just as irregularly as was noted for the agglutinins. The length of time during which they remain in the serum is difficult to determine without repeated tests on the same subjects; but one may be certain at least that they persist in some persons, vaccinated for the first time, for longer periods than has been usually supposed. In this connection Hage and Korff-Petersen<sup>2</sup> recently reported, using an extract antigen, that some persons may give fixation with from 0.01 to 0.025 c.c. antigen five to nine months after an initial antityphoid vaccination.

Repeated vaccination apparently favors this persistence of the complement fixing antibodies, inasmuch as nearly 70 per cent. of the thirteen cases in Group 1 still gave slight fixation of the complement. Unfortunately, the serums of two cases in this group were anticomplementary. The degree of fixation, however, was slight, only the maximum amount of antigen having been absorbed by the serum. In some instances a parallelism between the degree of fixation and the agglutination was evident, but no definite relationship could be established.

The conclusions from experimental work on animals that the complement fixing antibodies, which ordinarily develop later than the agglutinins in the blood serum, remain longer demonstrable are apparently not applicable to man. It would appear that the degree and persistence of these immune bodies is as irregular and as inconstant as was noted in the case of the agglutinins.

In the second group, containing thirty-seven subjects vaccinated with Gay-Claypole vaccine sediment, eighteen had received the sensitized vaccine sediment for the first time. The serums of those inoculated from seven to ten months previously showed, in 13.3 per cent. of the cases, fixation of the complement; the degree of the reaction

41. Hooker, Sanford B.: Preliminary Studies on the Antigenic Properties of Different Strains of *Bacillus Typhosus*, Proc. Soc. Exper. Biol. and Med., 1916, **13**, 139.

42. Garbat, A. L.: Studies in Typhoid Fever. Reference to a paper read before the American Association of Immunologists, Jour. Am. Med. Assn., 1916, **67**, 149.



was less marked than in Group 1. It is possible, however, that an additional 20 per cent. of serums can be added to the 13.3 per cent. which were not counted as positive on account of the anticomplementary action of the double or single serum controls. The total of 43.3 per cent. for fifteen serums would fairly correspond with the result obtained in Group 1.

We are at present unable to explain the high percentage of autotropic or anticomplementary serums in this group of subjects inoculated with sensitized vaccine. In so small a series they may, of course, be chance occurrences. Unfortunately, repeated tests were not possible, and prolonged exposure to higher temperature did not materially affect the serums. When reading Löwy's article several months after the tests were completed, we noted that he had already observed an analogous condition of the serums in rabbits vaccinated with sensitized living organisms. A separate experimental study, undertaken quite recently by one of use (K. F. M.) to explain the phenomenon, is now in progress.

The serums of three patients vaccinated two months ago still gave fixation with the polyvalent as well as with army antigen. As a rule, the serums of Group 2 gave just as good fixation with the polyvalent antigen as with the army and the extract antigens. Slight differences were noted, in two cases only, in the amounts of antigen bound by 0.1 c.c. of serum.

In nineteen subjects who had been repeatedly vaccinated with sensitized or army vaccine, about 50 per cent. of the serums gave positive complement fixation reactions. This series of cases supports the view already expressed that repeated vaccinations favor the persistence of fixing substances. To what extent the previous army vaccination had influenced the reactions in Group 2 is, of course, a matter of speculation.

Only a few points in connection with Group 3 need explanation. Of eighteen subjects with negative histories, one gave a decided complement fixation reaction and a marked agglutination. This case was therefore considered, as previously stated, a probable instance of unrecognized typhoid fever. The remaining seventeen serums gave complete hemolysis with the various antigens. Two of these (both from women) showed delayed hemolysis in the serum controls and with *B. coli* antigens.

Among ten subjects in Group 4, who had a definite history of typhoid fever, complement fixation was present in but one case, a woman who had had typhoid fever three and one-half years before. In another case a delayed hemolysis with four different antigens was observed on several occasions, when blood was collected for other

purposes. Our findings, therefore, are quite in accord with those reported in the literature.

Of seven subjects (Group 5) who, in addition to having had typhoid fever, had been vaccinated from six to eight months previous to the serum tests, five gave positive complement fixation reactions. One of them, who in January gave a fixation with 0.2 c.c., gave in June of the same year, with three entirely different antigens and five different serum samples collected from one to six days apart, the same or even better fixation. In this individual the agglutinins were still high.

Unlike the agglutinins, the difference between the percentages of complement fixation reaction following inoculations with army vaccine as compared with sensitized vaccine sediment is relatively small; and it is easily possible that in another series the relations would be reversed. Indeed, in rabbits Nègre and Löwy reported greater production of complement fixing antibodies from injections of living sensitized vaccine than from unsensitized heat or ether killed vaccines (Wright's or Vincent's). Ardin-Delteil, Nègre and Raynaud obtained similar results with human beings. Very little is known, however, about the immune substances (bactericidal and complement fixing antibodies) called forth by sensitized vaccine sediment. According to von Liebermann, Theobald Smith and others, there is serious doubt that any of the sensitized vaccines as prepared at the present time have any uniformity in antigenic power. Moreover, in the case of the sensitized vaccine sediment of Gay and Claypole it is possible that the chemical and mechanical destruction and subsequent extraction of the residue will further influence its antigenic properties.

The chart, it will be seen, strongly suggests that repeated vaccinations favor the persistence of complement fixing bodies.

#### THE RELATION OF THE AGGLUTININS AND COMPLEMENT FIXING ANTIBODIES TO THE TYPHOIDIN QUOTIENT

Elsewhere in this number of this journal it is shown by one of us that the typhoidin cutaneous test as measured by the typhoidin quotient, while it bears some relation to immunity, is exceedingly variable in its results. The subjects of these serologic experiments were the same as those for the series of typhoidin tests, the blood being drawn in each case at the time of performing the typhoidin inoculation. In Table 3 the average typhoidin quotients are compared with the agglutinin and complement fixing antibody averages in the five groups of subjects.

Inspection of this table shows a certain amount of relationship between the serologic findings and the typhoidin quotients. In all three columns there are low averages in the "negative history" group

and higher ones among the groups of supposedly typhoid immunes. The correspondence is far from exact, however, as is inevitable in a series of this size in which the figures of each group vary so much among themselves and in which the differences between the group averages, particularly among the typhoidin quotients, are not great. So that little can be said from the comparison other than that some relationship exists between all three tests and the typhoid experience of the subjects.

TABLE 3.—COMPARISON OF SERUM REACTIONS WITH TYPHOIDIN REACTION

Group	Number of Cases	Method of Vaccination	Time Interval	Average Agglutination Titration	Percentage of Complement Fixation	Average Typhoidin Quotient
1	12 A.* (13 C.F.)	Army vaccine, first time...	3 to 8 mo.	1:148	53.8	1.46
	11 A. (13 C.F.)	Army vaccine, repeated....	8 mo.	1:89	69	1.42
2	17 A. (18 C.F.)	Gay-Claypole sensitized sediment	2 to 10 mo.	1:32	44	1.4
	17 A. (19 C.F.)	Gay-Claypole sensitized sediment, repeated	2 to 8 mo.	1:59	47	1.48
3	18	Negative history.....	.....	1:30	5.5	1.4
4	10	Typhoid fever.....	3½ to 25 yr.	1:38	10	1.43
5	7	Typhoid fever and vaccinated	6 to 8 mo. before	1:59	71.4	1.56

\* The letter A. signifies agglutination; C.F., complement fixation.

## COMMENT

Hesitation has just been expressed in stating any very definite relationship between complement fixation, agglutination and the typhoidin cutaneous test. Still greater caution needs to be exercised before interpreting the findings of any of these procedures in terms of immunity. It should be clearly recognized that the only reason for attaching an immunity significance to one of these tests is the fact that they are positive (part of the time and in varying degrees) among classes of individuals who, experience has proved, are largely (not invariably) immune to typhoid fever. It has never been shown that the cases of typhoid fever subsequent to vaccination or a previous attack of the disease are those who react negatively to any of these tests. Moreover the low averages in all three columns of Table 3 (those who have had typhoid fever), if substantiated by sufficient



numbers of observations, would have to be regarded as distinctly limiting the value of these tests as indexes of immunity; because it is well known that most of those who have had typhoid fever even many years ago are highly immune.<sup>20</sup> There is nothing in our results, therefore, which is not in harmony with the long-accepted idea that the estimation of antibodies furnishes no accurate index of the power which the body possesses of defending itself against invasion by typhoid organisms. Thus, Garbat and Meyer<sup>43</sup> found that serums of rabbits immunized with sensitized cultures were highly protective (more so than animals treated with nonsensitized preparations), and yet this potent serum contained but small amounts of agglutinins and complement fixing antibodies.

We were unfortunately unable to carry out bacteriolytic experiments with the quantities of serum at our disposal. Neufeld and Hüne<sup>44</sup> found no definite relation between the complement deviating properties of the typhoid immune serum and the content of bacteriolytic amboceptors. But according to Wollstein<sup>3</sup> and Howell<sup>4</sup> the bactericidal properties of serums of persons immunized with Russell's vaccine behave in many respects similar to the complement fixing antibodies in our tests.

It is clear that too little is known about the serologic or cutaneous tests as indexes of typhoid immunity at the present time to make them worth anything when applied to individual cases with the idea of deciding about the need for prophylactic inoculation. Neither can they in their present development form the basis of reliable statistical researches to determine the relative merits of different types of vaccines. From the reviews of the literature, it is also evident that for further advances in the subject we must look to carefully controlled experiments with large numbers of observations. Our own series, while it is larger than the majority of those reported in the recent literature, and furnishes fairly definite information and suggestions on certain points, is too small to throw any light on some of the important questions involved.

#### CONCLUSIONS

Agglutination and complement fixation reactions have been studied in ninety-eight healthy individuals. Some of the subgroups are too small to warrant final conclusions, but the following points have a fair degree of probability:

43. Garbat and Meyer: Ueber Typhus Heilserum, *Ztschr. f. exper. Path. u. Therap.*, 1910, **8**, 1.

44. Neufeld, F., and Hüne: Untersuchungen über bactericide Immunität und Phagocytose, nebst Beiträgen zur Komplementablenkung, *Arb. a. d. k. Gsndtsamte.*, 1907, **25**, 201.

1. In comparison with army vaccine, sensitized vaccine sediment produces small amounts of agglutinins.

2. Complement fixing antibodies are found in about equal amounts in the serum of those who have had army vaccine and those who have received sensitized vaccine.

3. It has been shown anew that for individual cases serologic and allergic immunity tests in their present form are inconclusive as measures of typhoid immunity.

45. In addition to the references already given, the following will be found of interest:

Garbat, A. L.: The Complement Fixation Test in Typhoid Fever, Its Comparison with the Agglutination and Blood Culture Method, *Am. Jour. Med. Sc.*, 1914, **148**, 84.

Kilgore, Eugene S.: The Typhoidin Quotient, *THE ARCHIVES INT. MED.*, 1916, **17**, 25.

Liebermann, L., and Acél, D.: Ueber antigene Wirkung sensibilisierter und nicht sensibilisierter Blutzellen und Typhusbakterien, *Deutsch. med. Wchnschr.*, 1915, **41**, 965.

Smith, T.: The Underlying Problems of Immunization; reference in *Jour. Am. Med. Assn.*, 1916, **66**, 1740, and *Med. Rec.*, New York, 1916, **89**, 974.

Ardin-Delteil, Nègre and Raynaud: Recherches cliniques et experimentales sur la vaccinothérapie de la fièvre typhoïde par le virus sensibilisé de Besredka, *Ann. de l'Inst. Pasteur*, 1913, **27**, 644.

Force, J. N.: Institutional Vaccination Against Typhoid Fever, *Am. Jour. Pub. Health*, 1913, **3**, 750.

## d-GLUCOSE TOLERANCE IN HEALTH AND DISEASE\*

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In previous communications<sup>1</sup> from this laboratory descriptions have been given of apparatus devised for the purpose of making continuous intravenous injections at accurately controlled rates. The present paper deals with the application of this apparatus and the principles of timed intravenous injection to a clinical study of glucose tolerance in normal human individuals and in a series of pathologic conditions. In order that the work may appear in its proper relationship to that of previous writers the more important contributions to the subject may be reviewed.

It has long been known that sugars administered by mouth in sufficient amounts cause melituria, but an attempt to learn the size of the dose which would accomplish this was first made by Worm Müller<sup>2</sup> in 1884. Two healthy men on a diet free from carbohydrate received weighed quantities of glucose, lactose and saccharose and it was found that 50 gm. of glucose, 50 gm. of saccharose or 100 gm. of lactose produced just a trace of reducing substance in the urine, while larger doses caused severe melituria.

Hofmeister<sup>3</sup> in 1889 conducted a more elaborate investigation with dogs. The sugar was fed by mouth and it was found that the maximum dose that could be given and just fail to cause sugar to appear in the urine was very constant for the individual dog, although widely different for different dogs. This dose he called the "assimilation limit," a term that has since been widely employed. Hofmeister is also the author of the term "tolerance," as applied to sugars, which he used in the following connection:<sup>4</sup>

The diabetic of the milder type on a strictly regulated meat diet has a certain tolerance for sugars and starches, inasmuch as he only passes a saccharine urine when such substances are administered in excess of a definite limit.

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1. Woodyatt, Sansum and Wilder: Jour. Am. Med. Assn., 1915, **65**, 2067.

2. Müller, Worm: Arch. f. d. ges. Physiol., 1884, **34**, 576.

3. Hofmeister: Arch. f. exper. Path. u. Pharmakol., 1889, **25**, 240.

4. Der Diabetiker, leichterer Form zeigt, bei streng gehandhabter Fleischkost, eine gewisse Toleranz für Zucker und für Amylacen, insofern er nur bei Zufuhr derselben über eine gewisse Grenze hinaus Zuckerhaltigen Harn liefert.



It does not appear that he drew a distinction between tolerance and assimilation limits, and since this time assimilation limits, as measured by Hofmeister's method, have been interpreted as tolerance limits.

In 1895 Linossier and Roque<sup>5</sup> confirmed the observations of Worm Müller and Hofmeister, but proposed, as a truer measure of the assimilability (*Nutzwert*) of a sugar, the coefficient obtained by dividing the quantity of sugar excreted by the quantity given. The constants as found by these authors were variable, even for the same sugar; but in 1898 Gilbert and Carnot<sup>6</sup> obtained a fairly constant ratio of excretion to administration by giving the sugar intravenously. Inconstant proportions occurred when the injected amounts were small, but with doses between 2.5 and 10 gm. per kilogram of body weight, the ratio of grams excreted and grams injected was always about 40 to 100.

The intravenous injection of sugar is an old procedure and it has frequently been observed when different sugars were given in this way that some were excreted more freely than others. On the basis of such observations conclusions have been drawn regarding the relative utilizabilities of the various sugars. But whether these conclusions are justified on the basis of the experiments made is open to question because of the excessive variations that occur in the results of different writers and even in those of the same observer. Pavy,<sup>7</sup> for example, after injecting the same sugar (glucose) in doses of 0.25 gm. per kilogram of body weight in a series of rabbits, recovered in the urine of one as little as 3.1 per cent. and from another as much as 39.6 per cent. More consistent results with intravenous injections were obtained by McGuigan and Mathews,<sup>8</sup> who gave dilute, 0.5 per cent., sugar solution in such a manner that 5 c.c. entered every five minutes. In some cases these injections were continued for as long as seventy-seven minutes and conclusions concerning the relative ease of oxidation of the sugars were drawn from their time of appearance in the urine. Previous to this Doyon and Dufourt<sup>9</sup> had discovered that the *rate* of intravenous administration influenced the amount of sugar excreted and it would appear that the variation in the results obtained by the earlier writers was due to their failure to appreciate the importance of controlling the rate of injection. Doyon and Dufourt used dogs and gave to each 2 gm. of glucose per kilogram of body weight, "en un temps variable mais avec une vitesse toujours uniforme." They do not explain how they maintained such constant injection rates and simply report the

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5. Linossier and Roque: Arch. de méd. expér. et d'anat. path., 1895, **7**, 228.

6. Gilbert and Carnot: Compt. rend. Soc. de biol., 1898, **50**, 330.

7. Pavy: Jour. Physiol., 1899, **24**, 479.

8. McGuigan and Mathews: Am. Jour. Physiol., 1907, **19**, 175.

9. Doyon and Dufourt: Jour. de physiol. expér., 1901, **3**, 703.

result of one typical experiment which disclosed that 98.64 per cent. of the sugar was retained if the duration of the injection was made eighty minutes, while only 85.62 per cent. remained in the body when the same quantity was injected in fifteen minutes.

In 1905 Blumenthal<sup>10</sup> pointed out that the utilization of sugar by the tissues is expressible only in terms that apply to velocities, for example, as unit weight of sugar, per unit weight of tissue, per unit weight of time. The rate at which sugar is brought to the tissues must therefore be controlled if it is desired to measure its rate of utilization. This necessitates precise knowledge of the rate at which it enters the blood; but when a sugar is given by any method entailing absorption as a preliminary to its entrance into the blood, the latter rate is subject to uncontrollable variations. The most nearly exact method of determining sugar tolerance must therefore consist in direct intravenous injection. These principles he applied in a series of experiments and found (1) that glucose could be rapidly injected into rabbits in doses of about 0.85 gm. per kilogram of body weight without causing melituria, but if this dose was only slightly exceeded or if within a short time after the first dose a second injection was made, intense melituria would result; (2) that a dose of sugar could be selected which could be repeatedly injected at fifteen minute intervals for indefinite periods and always just fail to appear in the urine. From these observations Blumenthal concluded that the tissues are able to remove sugar from the blood at a rapid rate up to a certain limit, that is, until they have become "saturated," but no inference concerning utilization can be drawn from the size of the dose necessary to cause saturation or the rate at which this dose is absorbed. Such a dose represents simply a saturation limit (*Sättigungsgrenze*). On the other hand, that dose whose repeated injection at fifteen minute intervals just fails to produce melituria gives a measure of utilization, a utilization limit (*Ausnutzungsgrenze*). For glucose in rabbits this was found to be between 0.15 and 0.325 gm. per kilogram of body weight per fifteen minutes, that is, from 0.6 to 1.2 gm. per kilogram per hour.

In 1906 Comessatti<sup>11</sup> used the Blumenthal method and demonstrated that sugar assimilation was increased by exercise. During rest, doses of 0.2 gm. per kilogram per fifteen minutes led to melituria in two rabbits, while after exercise in a treadmill doses of 0.25 gm. given in the same manner caused no glycosuria. In 1909 Loeb and Staddler,<sup>12</sup> with the same method, obtained figures in resting rabbits which were lower than those reported by Blumenthal (0.09 and 0.16

10. Blumenthal: Beitr. z. chem. Phys. u. Path., 1905, **6**, 329.

11. Comessatti: Beitr. z. chem. Physiol. u. Path., 1906, **9**, 67.

12. Loeb and Staddler: Arch. f. exper. Path. u. Pharmakol., 1914, **77**, 326.



gm. per kilogram of body weight per fifteen minutes, that is 0.36 and 0.64 gm. per kilogram per hour).

There can be no doubt of the correctness of the principle stated by Blumenthal or of the insuperable difficulties which lie in the way of accurate measurement of the power of the tissues to utilize sugar when methods are used in which it is impossible to control the rate of entry of sugar into the blood. If the sugar is given by mouth, as in the Hofmeister procedure, the rate of absorption will depend on variable factors entirely beyond control, and when the subcutaneous or intraperitoneal routes are chosen the same difficulty is encountered. A tolerance limit obtained by such methods is at best only the resultant of absorption and utilization, and yet since the appearance of Blumenthal's article writers other than those quoted above have failed to appreciate the soundness of his views and continue in their adherence to absorption methods.

In clinical studies it is a common practice to give by mouth 100 gm. of d-glucose dissolved in 200 c.c. of water or lemonade and to speak of a low tolerance if this amount of sugar leads to melituria. If no melituria results when 150 or 200 gm. are fed, tolerance is considered high, but the test is by no means reliable, as many normal persons can tolerate any quantity of glucose which they can be made to take and retain, a fact which has been confirmed strikingly by the experiments of Taylor and Hutton.<sup>13</sup> Nevertheless, such tests have been applied in a wide variety of clinical conditions, especially in diseases of the "glands of internal secretion," and have formed the basis of extensive theory. Other methods for measuring tolerance have received little attention from clinicians. Subcutaneous injections of sugar have been made (Voit,<sup>14</sup> Achard<sup>15</sup> and others), but the pain and danger of infections which they involve have limited their general use. Intravenous sugar injections, while employed for therapeutic purposes (Kausch<sup>16</sup> and others) have not been used in clinical tolerance studies and the attention of clinicians seems not to have been directed to the contribution of Blumenthal.

But while Blumenthal's statement of the principles governing accurate measurement of sugar tolerance is sound and demands recognition, his technical application of these principles is capable of improvement. This is particularly true if the method is to be applied in clinical investigation. Blumenthal's method involves repeated injections at fifteen minute intervals, and the technical difficulties of performing these on time, and with assurance that no leakage occurs, are

13. Taylor and Hutton: *Jour. Biol. Chem.*, 1916, **25**, 173.

14. Voit, F.: *Deutsch. Arch. f. klin. Med.*, 1897, **58**, 523.

15. Achard, cited by Lepine: *Le diabète sucré*, Paris, 1909, p. 234.

16. Kausch: *Deutsch. med. Wchnschr.*, 1911, **37**, 8.



very material, particularly in patients with small superficial veins. The Blumenthal procedure is furthermore insufficient to accomplish that which Blumenthal's principles demand, namely, a perfect control of the rate of administration. His injections, repeated every fifteen minutes, are not continued at a uniform rate over the fifteen minutes, but are given rapidly at the beginning of each period. Under these conditions it is clear that in the first part of each fifteen minute period, that is, during the time actually occupied by the injection, the concentration of sugar in the blood must rise as a wave and in the interval between the injections the concentration must fall. If now the injection is made more rapidly in one case than another, the crest of the wave produced will be higher, and such a wave may overflow the kidney threshold and result in melituria, even though the tolerance for the fifteen minute period is by no means exceeded. Under these conditions it is incorrect to assume that a melituria indicates that the rate of utilization for the period has been overstepped. That this objection to Blumenthal's procedure is sound is shown by figures for the tolerance of rabbits published by Blumenthal,<sup>10</sup> by Comessatti<sup>11</sup> and by Loeb and Staddler,<sup>12</sup> all of whom used the same method. One rabbit of Blumenthal's showed a tolerance of 0.325 gm. per kilogram of body weight per fifteen minute period, while one of Loeb and Staddler's was as low as 0.09 gm. per kilogram per fifteen minutes; and Blumenthal's own results ranged, as stated above, from 0.15 to 0.325 gm. per kilogram per fifteen minutes, a variation of over 100 per cent. It is probable that the higher figures obtained by these authors were more nearly correct than the lower ones and that the latter occurred when the injections were made too rapidly.

In order to avoid periodic waves of sugar concentration and to give no opportunity for melituria to occur until the rate of administration at all times surpasses that of utilization, it is clearly advisable to discard intermittent or discontinuous injections in favor of continuous administration at constant rates. The latter procedure was adopted in the present study, and for the purpose of giving continuous injections use was made of the apparatus referred to above. This apparatus consists of a small pump which is driven by an electric motor. The stroke of the pump may be made very short and the number of strokes per minute raised to forty or sixty, so that the stream pumped is practically continuous. Such pulsations as occur are furthermore almost obliterated by the elasticity of the rubber tubing that connects the pump with the needle in the patient's vein. The rates of injection are easily controlled and accurately regulated by means of a rheostat.

By the use of this instrument it is a simple matter to find that rate of injection which just equals the rate of utilization or the tolerance limit.

The limits for d-glucose of different dogs and rabbits tested in this way were nearly constant and approximated Blumenthal's higher figures, as might be anticipated. Reports of these animal experiments will appear in other communications, as the present paper is limited to the results obtained in human subjects.

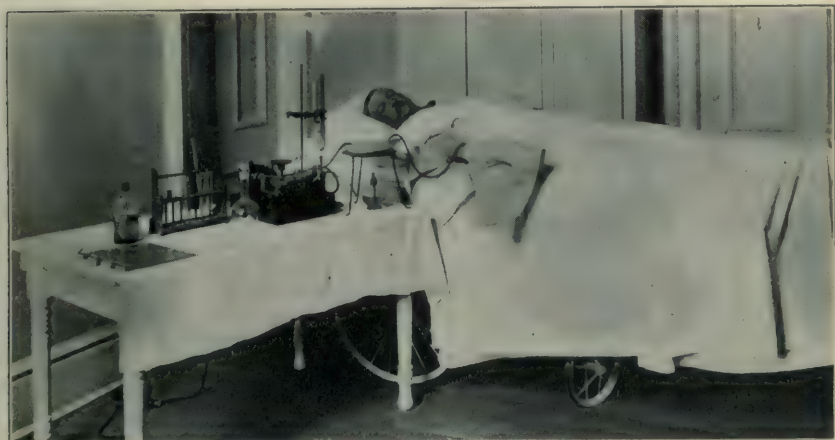
#### PROCEDURE IN TESTING THE d-GLUCOSE TOLERANCE OF MAN

It is to be anticipated that the intravenous tolerance limits for glucose will vary with a number of physiologic factors capable of influencing the basal metabolism. If the tolerance is expressed as grams of glucose per unit of body weight per unit of time, then sex, age, the proportion of the different types of tissue entering the body structure, the relative proportion of fat, glycogen and protein in the respective tissues, the ratio of surface to mass, the state of the activity of the muscles, etc., all require due consideration. In the present study it was found that in health both men and women showed the same tolerance. The influence of age was minimized by confining the work to adults between the ages of 20 and 45. (One child of 14 is reported.) The subjects chosen were with one exception in an average state of nutrition. All had been on a general mixed diet for several days before examination and none had partaken excessively of carbohydrates. On the day of the test no food was given by mouth and the sugar injection was commenced in the afternoon between 2 and 4 p. m. Uniform conditions of rest were secured by keeping the subjects in bed on the day of the test, and finally, in order to minimize possible delays in the excretion of glucose, once the tolerance limit had been exceeded, as well as to study the effects of different rates of glucose injection on the diuresis, water was given by mouth in amounts equivalent to the amount of fluid necessary to administer during the injection, in such a way that the subject would receive during each hour of the foreperiod an amount of water equivalent to the quantity which he was to receive by vein in each hour of the test.

*Material.*—The d-glucose employed in earlier experiments was a preparation of Merck labeled "purissimum." Later a high grade granular commercial dextrose (Argo corn sugar) was purified in the laboratory by decoloration with animal charcoal and repeated recrystallization from alcohol. The final product was dissolved in freshly distilled water (prepared with such precautions as are employed for salvarsan administration) to make from 16 to 20 per cent. solutions. (The molecular weight of glucose being 180, the gram molecular solution is 18 per cent.) Such a solution is sufficiently hypertonic to avoid hemolysis and has been found for other reasons to be the best standard solution to employ for the present purposes. In earlier experiments

solutions of greater strength were employed, with a view to keeping the volume injected small, but the opportunity for slight variations in the injection rate was thereby correspondingly increased and on this account the 18 per cent. solutions were adopted. The solutions showed only pale straw color when observed in flasks of two liter volume, smaller volumes appearing colorless. The solutions were divided in one and two liter flasks which were sterilized in the autoclave. The titer of each was determined by a polariscope directly before injection.

In the entire course of this work, in which the solutions were injected at rates slightly above and below the tolerance limit, no chills, shock or other general reactions were encountered. In two cases thrombosis of the vein used for the injection resulted. In one of these



Illustrating apparatus and method of making the glucose injection.

the thrombus was limited to an inch of the vein, in the other it extended to the axilla, but in neither did it result in a bad effect. It is probable that the thrombus formation in the most extensive of these cases was favored by trauma, as the subject of the test refereed a football game on the day after his injection.

*Technic of Injection.*—As previously stated, use is made of the injecting machine developed in this laboratory and described elsewhere. A buret to serve as a reservoir, together with the necessary rubber tubing, are sterilized by autoclave. The parts of the pump which come into contact with the fluid are removed and boiled. The apparatus is then assembled in the manner illustrated, the glucose solution is poured into the buret and all air expelled from the tube and pump.



The initial rate for injection is purposely chosen below what is thought to be the velocity of assimilation; for example, if the subject is apparently normal, it is assumed that a rate of 0.7 gm. per kilogram of body weight per hour will be tolerated, and this rate is given. If the patient weighs 75 kg. and the glucose solution, as determined by polariscope, is 20 per cent., this would mean an injection of  $0.7 \times 75 \times 5$ , or 262.5 c.c. of a 20 per cent. solution per hour, or 4.37 c.c. per minute. The machine is set to deliver the solution at this rate, the needle is introduced into a suitable vein and the injection begins.

This initial injection rate must be maintained long enough to permit a thorough saturation of the tissues before the urine is collected and tested for sugar. This requires from twenty to thirty minutes, as has been found by experiment. If the injection rate only slightly exceeds the rate of utilization, glycosuria will occur within this time; if it does not, no glycosuria will occur even after several hours of continuous injection. This latter statement is based on experiments with dogs. For this reason the first collection of urine is made about thirty minutes after the beginning of the experiment. One cubic centimeter of urine is added to 5 c.c. of heated Haines' solution, the mixture boiled for ten seconds and cooled and the absence of reduction is taken to indicate that the tolerance of the patient lies above the rate of injection. The injection is then accelerated and continued for thirty minutes at this higher rate, for example, at 0.8 gm. per kilogram per hour in the case under consideration. The urine is then tested again and by gradually stepping up the rate of injection, 0.1 gm. per kilogram per hour at a time, that rate can be found which first causes glycosuria and the rate just preceding this is accepted as the tolerance limit.

#### RESULTS

*d-Glucose Tolerance in Health.*—Four young adults, two men and two women, were chosen for the tests of normal glucose tolerance. All of them had been working in the medical college or the hospital and were known to be in good health. On the day preceding the test they were put to bed and subjected to the experimental conditions outlined above. The results in all these cases were the same. Glucose injected at the rate of 0.8 gm. per kilogram of body weight per hour caused no glycosuria, but in each case sugar appeared in the urine when the injection rate was 0.9 gm. per kilogram per hour. From this it would appear that the tolerance limit for d-glucose of normal men and women is practically constant and that it equals a rate of approximately 0.85 gm. per kilogram of body weight per hour of time. For a 75 kg. man such an injection would represent the administration of 63.75 gm. per hour.

*Tolerance in Diseases of the Pancreas.*—Three patients, one with organic disease of the pancreas and the other two with suspected mild diabetes mellitus, were tested for their glucose tolerance. In both a low limit was demonstrable. The first suffered from a chronic interstitial pancreatitis, a diagnosis which was confirmed by subsequent operation. His tolerance limit lay below 0.7 gm. per kilogram of body weight per hour. The second patient had recently shown a trace of sugar in the urine at a life insurance examination, but had been sugar free since then. His tolerance lay below 0.5 gm. per kilogram per hour. The third was similar to the second, with a tolerance of 0.4 gm. per kilogram per hour; but since the state of his nutrition and other factors besides the pancreatic disease were not strictly comparable with the controls, some reserve is expressed as to the significance of this result.

*Tolerance in Diseases of the Thyroid.*—Five patients with exophthalmic goiter, two men and three women, have been tested for their glucose assimilation and in all the tolerance limits were reduced. Two of these patients had severe general symptoms and these cases showed glycosuria when glucose was administered at the rate of 0.5 gm. per kilogram per hour. Two milder cases gave a limit exceeded by the administration of 0.6 gm. per kilogram per hour, and the fifth, a still milder case, had a tolerance between 0.6 and 0.7 gm. per kilogram per hour. It would appear, therefore, that a depressed glucose tolerance is a fairly constant accompaniment of exophthalmic goiter. Such results are in harmony with the observation of alimentary glycosuria in exophthalmic goiter made by Kraus and Ludwig,<sup>17</sup> Chvostek,<sup>18</sup> Falta<sup>19</sup> and many others and with the glycosurias which may be produced in animals and normal persons by feeding thyroid tablets (Ewald,<sup>20</sup> Dale,<sup>21</sup> Denning,<sup>22</sup> von Noorden<sup>23</sup> and others). On the other hand, a typical case of myxedema was found to have a normal tolerance, by the intravenous method, a result which is not in agreement with the observations of those who use the alimentary tests and find high assimilation limits in this disease, suggesting that a delay in the rate of absorption is accountable for their findings.

*Tolerance in Disease of the Hypophysis.*—Two cases of acromegaly have been studied, one case of suspected gigantism and two with dys-

17. Kraus and Ludwig: Wien. klin. Wchnschr., 1891, **4**, 898.

18. Chvostek: Wien. klin. Wchnschr., 1892, **5**, 17.

19. Falta: Die Erkrankungen der Blutdrüsen, Berlin, 1913, p. 65.

20. Ewald: Die Erkrankungen der Schilddrüsen, Myxödem und Kretinismus, Ed. 2, Leipzig, 1909.

21. Dale: Brit. Jour. Dermat., 1894, **6**, 177.

22. Denning: München. med. Wchnschr., 1895, **42**, 389.

23. Von Noorden: Ueber den Einfluss der Schilddrüsenbehandlung auf den Kohlehydratstoffwechsel, Berl. klin. Wchnschr., 1897, **34**, 518.

pituitarism, showing the Fröhlich syndrome. The glucose tolerance of all these cases was found to lie close to the normal limits. Three showed a bare trace of sugar in the urine after the injection of 0.8 gm. per kilogram per hour and all gave a definite glycosuria after receiving 0.9 gm. per kilogram per hour. These results are in disagreement with those obtained when the oral administration is used (Cushing,<sup>24</sup> Bondi,<sup>25</sup> Falta<sup>19</sup>) and suggest, as in the case of myxedema, that the increased tolerance of such patients for sugar given by mouth is due rather to a retarded absorption from the bowel than to any anomaly of the intermediate metabolism, as certain writers have assumed.

*Tolerance in Cirrhosis of the Liver.*—A normal d-glucose tolerance limit was found in a case of alcoholic cirrhosis.

#### SUMMARY

Adopting the premise previously suggested by Doyon and Dufourt, and notably Blumenthal, that glucose tolerance must be dealt with as a velocity, that is, in terms of the number of grams of glucose which may be brought into the tissue per unit of body weight per unit of time without causing an abnormal overflow of glucose into the urine, we injected from 16 to 20 per cent. solutions of pure d-glucose intravenously at different uniform rates by means of the motor driven quantitative injection pump, which has been described in other reports from this laboratory. Observations have been made on rabbits, dogs, and normal resting men and women of average size, weight and nutrition. These experiments indicate that glycosuria appears when the rate of injection lies above 0.8 gm. and below 0.9 gm. per kilogram of body weight per hour.

It is realized that variations from these limits may be ascribed, *a priori*, to any one of several factors, namely, (1) variation in the ability of the colloids of the body at large, including those of the kidney, to hold glucose in a state of adsorption or its equivalent (variations in the threshold for glucose); (2) the variation in the rate at which glucose may be entering the cells from endogenous sources, notably glycogen and protein, but especially the former, since endogenous supplies of glucose to the cells must be regarded as superadding themselves to the glucose supplied by injection; (3) to variation in the rate at which glucose can be utilized within the cell, the utilization in this instance being taken to imply the sum of those processes by which glucose may undergo a chemical change into some substance other than glucose.

The purpose of the present report is to record objectively experi-

24. Cushing: The Pituitary Body and Its Disorders, Philadelphia, 1911.

25. Bondi: Arch. f. exper. Path. u. Pharmacol., 1910, **63**, 347.



mental findings without entering into detailed considerations of the physiologic factors which have been concerned.

Observations have been made of the intravenous glucose tolerance limit in the following clinical conditions: chronic interstitial pancreatitis, one case; very mild diabetes, two cases; thyroid disease, six cases, five representing different grades of symptoms of hyperthyroidism and one a typical instance of myxedema; hypophysis disease, five cases giving clinical pictures which would be designated as such; gigantism (?), one case; Frölich syndrome, two cases (one with diabetes insipidus and one without); acromegaly two cases; and cirrhosis of the liver, one case.

Many questions which arise as to the variations of the intravenous glucose tolerance as the result of variations in the state of nutrition, the amount of stored glycogen, the character of the previous diet, the water and salt balance of the body, etc., will be further discussed in other reports from this laboratory.

#### CONCLUSIONS

The following conclusions would appear to be justified:

1. Normal resting adult men and women of average size, weight and nutrition begin to excrete abnormal quantities of glucose in the urine when the injection rate into the vein is above 0.8 gm. and below 0.9 gm. per kilogram of body weight per hour.

2. Cases of nondiabetic pancreas disease may evince a lowered tolerance for glucose when measured by this method even when administration of glucose by the alimentary route gives no definite evidence of such diminution. The same is true of very mild cases of diabetes mellitus. In these cases it may be fancied that the lowered tolerance arises from a diminished utilization.

3. Cases with increased thyroid function show a uniformly diminished intravenous glucose tolerance. In such cases it is suspected that this effect may be ascribed to an increased glycogenolysis, which is tantamount to an endogenous glucose supply to the cells superimposed on the intravenous injection, or, what amounts virtually to the same thing, a decreased ability on the part of the body to build up glycogen, since increased glycogenolysis implies the disbalance between the synthesis and catalysis of glycogen in favor of the latter. On the other hand, a case with decreased thyroid function (myxedema) showed no increased glucose tolerance, but appeared to have a normal tolerance, excreting sugar promptly when this was injected at a rate faster than 0.9 gm. per kilogram of body weight per hour.

4. In none of the cases of hypophysis disease has there been any increase of the glucose tolerance above the normal limits, but on the

contrary, a tendency toward decrease, which would seem to imply that increases of glucose tolerance in such cases, following alimentary administration of glucose, are due to delays in absorption rate rather than to changes in the intermediate metabolism.

5. The d-glucose tolerance of an advanced case of alcoholic cirrhosis of the liver was found to be normal.

6. The determination of glucose tolerance by the method described is a practical clinical procedure, more accurate than those methods which depend on absorption from a local site as a preliminary to the entrance of glucose into the blood and giving more constant results than have been obtained by any other technic involving intravenous injections.

#### PROTOCOLS AND CASE HISTORIES

The protocols of these experiments, the case records and a composite chart of the results (Fig. 2) are appended.

CASE 1.—B. T., a healthy woman, aged 25, weight 42.7 kg., rested in bed on the day of the test.

TABLE 1.—GLUCOSE SOLUTION, 18 PER CENT. BY POLARISCOPE

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope	Total Gm.
0.8	3.16	30	0		
0.9	3.56	30	++	0.4	0.96
Subsequent loss.....					0.75+
Total glycosuria.....					1.71+

Tolerance lies between 0.8 and 0.9 gm. per kilogram per hour.

CASE 2.—P. G., a healthy woman, aged 21, weight 60 kg., rested in bed on the day of the test.

TABLE 2.—GLUCOSE SOLUTION, 18 PER CENT. BY POLARISCOPE

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope	Total Gm.
0.8	4.45	38	Trace*	0.16	0.77
0.9	5	20	+	0.33	1.45
Subsequent loss.....					?
Total glycosuria.....					2.22+

\* During the last three minutes of injection at the 0.8 gm. rate the pump accidentally accelerated. This accounts for the trace of sugar found in the urine. The tolerance, therefore, probably lies above 0.8 but below 0.9 gm. per kilogram per hour.

Diagnosis	*Case No.	Tolerance for d-Glucose	Grams Per Kilo. Hour
Normal .....	1		0.85
Normal .....	2		0.80
Normal .....	3		0.85
Normal .....	4		0.85
Pancreatitis .....	5		0.70
Mild diabetes .....	6		0.50—
Mild diabetes .....	7		0.40
Hyperthyroidism .....	8		0.60—
Hyperthyroidism .....	9		0.55
Hyperthyroidism .....	10		0.55
Hyperthyroidism .....	11		0.65
Hyperthyroidism .....	12		0.60
Myxedema .....	13		0.85
Hypophysial disease.....	14		0.80
Hypophysial disease.....	15		0.80
Hypophysial disease.....	16		0.80—
Hypophysial disease.....	17		0.75
Hypophysial disease.....	18		0.85
Cirrhosis of liver.....	19		0.80

\* Numbers refer to case histories.



CASE 3.—W. S., a healthy man, aged 35, weight 73.2 kg., rested on the day of the test.

TABLE 3.—GLUCOSE SOLUTION, 40.91 PER CENT.

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscopes	Total Gm.
0.85	2.38	32	Trace	0.23	0.15
0.9	2.68	30	+	0.86	1.12
Subsequent loss.....					0.76
Total glycosuria.....					2.03

Tolerance lies very close to 0.85 gm. per kilogram per hour.

CASE 4.—J. V., a healthy man, aged 25, weight 75.45 kg., rested in bed on the day of the test.

TABLE 4.—GLUCOSE SOLUTION, 19.75 PER CENT. BY POLARISCOPE

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscopes	Total Gm.
0.8	5.1	30	0		
0.9	5.73	30	+	0.64	1.66
Subsequent loss.....					1.02
Total glycosuria.....					2.68

Tolerance lies between 0.8 and 0.9 gm. per kilogram of body weight per hour.

CASE 5.—Diagnosis, chronic pancreatitis. J. W., aged 45, was admitted to the service of Dr. J. B. Herrick at the Presbyterian Hospital on Nov. 6, 1915, with complaints of jaundice and intense itching. He gave a history of syphilis in 1888, for which he received treatment, herpes zoster in 1911, and in 1912 jaundice without pain, fever or chills, which lasted two weeks. In August, 1915, he again became jaundiced, this time with cold sweats, a chill and fever. There was no pain and the fever disappeared, but the jaundice continued. The patient lost in weight from 138 to 124 pounds.

Examination disclosed an intense icterus. The gallbladder was barely palpable, but not tender. The temperature was recorded as 100.6 on the day of admission, but remained normal for the next two weeks. The urine showed a large amount of bile, but no albumin or sugar. The stools were mushy, clay colored, gave a strongly positive Weber test for blood, and contained fat in abundance and some starch. The stomach contents after Ewald's test breakfasts showed no blood and were of normal acidities. Duodenal contents, aspirated by means of an Einhorn tube, contained blood and ferments, but no bile.

A diagnosis of organic disease of the head of the pancreas was made, which was confirmed by operation on Dec. 6, 1915. The patient showed marked

improvement following operation, gaining 12 pounds in weight. A second operation was made on Jan. 22, 1916, and the mass in the head of the pancreas was found to be smaller. Probable diagnosis was made of ulcer of the duodenum with secondary chronic pancreatitis and obstructive jaundice.

An intravenous glucose tolerance test on this patient made Nov. 22, 1915, gave the results shown in Table 5. The weight of patient was 54.3 kg.

TABLE 5.—GLUCOSE SOLUTION, 20.25 PER CENT. BY POLARISCOPE

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope	Total Gm.
0.2	0.89	30	0		
0.5	2.24	70	0		
0.7	3.13	82	Trace	0.1	0.25
0.9	4.02	35	++	0.66	2.00

The threshold of tolerance lies at or very close to 0.7 gm. per kilogram of bodyweight per hour, which is slightly below normal.

CASE 6.—Diagnosis, mild diabetes. R. D., a physician, aged 33, showed a trace of sugar in the urine in a recent examination. In one twenty-four hour specimen this had amounted to 0.2 per cent. He had suffered from an attack of appendicitis and underwent an operation in 1911. Two sisters had died of tuberculosis and one brother was obese, weighing 225 pounds.

The patient was a tall, muscular man. The tonsils were small and cryptic. There were a few fillings in the teeth and the gums were retracted at the roots of the left upper molars. The thyroid was not enlarged. A urinalysis was negative.

An intravenous glucose tolerance test was made and resulted as shown in Table 6. The weight of the patient was 83.2 kg.

TABLE 6.—GLUCOSE SOLUTION, 21.94 PER CENT. BY POLARISCOPE

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope	Total Gm.
0.5	3.16	80	++	0.69	0.53
Subsequent loss.....					1.19+
Total glycosuria.....					1.72+

The threshold of tolerance lies well below 0.5 gm. per kilogram of bodyweight per hour. The urine was only collected for one hour after the injection stopped.

CASE 7.—Diagnosis, mild diabetes. W. K., aged 49, was admitted to the service of Dr. Billings at the Presbyterian Hospital on June 23, 1916. His complaints were aching pains in the right thigh and the suspicion of diabetes.

He gave a history of excellent health up to 1910, when sugar was found in his urine. At that time he was losing weight, had excessive thirst and hunger and polyuria. He was placed on a restricted diet and was soon apparently normal. During the last year he has had rather frequent urination and for the

last three months has suffered from pains in the right thigh and a feeling of weakness in the legs on ascending stairs.

Examination showed a well-nourished man of average height. The teeth were badly discolored and decayed and the Roentgen ray revealed a chronic abscess at the root of one molar. The gums were retracted. A slight systolic murmur was audible at the apex. In all other respects the examination was negative.

Urinalysis of twenty-four-hour specimens showed 1,500 to 1,800 c.c., with specific gravities of 1.010 and 1.013. No reduction of Haines' solution was obtained and there was no evidence of diacetic acid or acetone.

An intravenous d-glucose tolerance test was made on June 26 with results as shown in Table 7. The weight of the patient was 75.4 kg.

TABLE 7.—GLUCOSE SOLUTION, 16.4 PER CENT. BY POLARISCOPE

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope	Total Gm.
0.3	2.29	80	0		
0.5	3.06	80	+	0.5	2.225

The threshold of tolerance lies between 0.3 and 0.5 gm. per kilogram of body weight per hour.

CASE 8.—Diagnosis, hyperthyroidism. M. R., a girl, aged 19, complained on admission of goiter, nervousness, dyspnea, palpitation, and edema of the feet. The trouble began as nervousness eighteen months ago. Six months later the thyroid enlarged and the eyes became prominent, and before admission the right lower jaw had often been swollen and painful. Menstruation had been normal.

On examination the patient presented a marked exophthalmos, a fairly large goiter of uniform, firm consistence over which a double murmur could be auscultated, a fine tremor of the tongue and fingers and a pulse ranging from 120 to 140. Adenoids and tonsils were hypertrophic. In the tonsils were deep crypts which contained pus. There were several broken-down teeth at the roots of which abscesses were demonstrable.

Blood counts revealed a moderate anemia and a mononucleosis of from 40 to 46 per cent. Urinary examinations showed an albuminuria of less than 0.1 per cent., a few casts, but no erythrocytes and no sugar.

An intravenous glucose tolerance test was made on this patient with results as shown in Table 8. The weight of the patient was 70 kg.

TABLE 8.—GLUCOSE SOLUTION, 21.8 PER CENT. BY POLARISCOPE

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope	Total Gm.
0.6	3.21	69	+	0.267	1.92
Subsequent loss.....					2.12
Total glycosuria.....					4.04

The threshold of tolerance lies below 0.6 gm. per kilogram of body weight per hour.



CASE 9.—Diagnosis, hyperthyroidism. E. L., a woman, aged 32, was admitted to the service of Dr. B. W. Sippy at the Presbyterian Hospital on Jan. 16, 1916, with complaints of goiter, bulging eyes, palpitating heart, nervousness and weakness. The family history was remarkable in that all the female members of the mother's side, including the grandmother, had large goiters. None had suffered from symptoms of exophthalmic goiter.

The patient had no goiter until the onset of the present trouble, but was always nervous. She had measles at 9, and had suffered from catarrh of the nose and throat from her eighteenth year. At 25 came an acute attack of tonsillitis. Her present illness commenced two months before admission with painful swelling of the thyroid. The pain disappeared in a week, but the swelling persisted; exophthalmos, palpitation and tremors appeared; she grew very nervous and lost weight rapidly.

Examination revealed a young woman in a fair state of nutrition. Exophthalmos was extreme and von Graefe, Möbius and Stellwag signs were present. The goiter was of medium size and of a uniformly firm consistence. A systolic bruit was heard over it. Submanubrial dullness was 7 cm. wide (thymus?). The heart was normal in size, the pulse 112 and regular. The leukocyte count was 8,500 with 45 per cent. mononuclears. The urine was normal on repeated examination.

An intravenous sugar tolerance test was made with results as shown in Table 9. The weight of the patient was 62.3 kg.

TABLE 9.—GLUCOSE SOLUTION, 17.6 PER CENT. BY POLARISCOPE

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope	Total Gm.
0.5	2.95	45	0		
0.6	3.54	46	+	0.5	3.23
Subsequent loss.....					3.41
Total glycosuria.....					6.64

Tolerance lies between 0.5 and 0.6 gm. per kilogram per hour.

CASE 10.—Diagnosis, hyperthyroidism. A. S., a man, aged 41, was admitted to the service of Dr. C. B. Davis of the Presbyterian Hospital on Aug. 16, 1915, with complaints of goiter, prominence of the eyes, palpitation, nervousness and loss of weight. His disease began in 1903 with thyroid swelling and nervousness. Exophthalmos occurred in 1904 and until 1905 he was very ill. Then followed a period of improvement, lasting until 1913, but after 1913 he suffered from extreme nervousness and palpitation and has lost twenty-five pounds in weight. Sugar has been found in the urine on several occasions.

When examined the patient presented the typical appearance of exophthalmic goiter. He was very thin, very weak and highly nervous. Exophthalmos was extreme, and von Graefe, Möbius and Stellwag signs were positive. The tumor of the neck was large and of a uniformly hard consistence. Over it was heard a systolic, diastolic bruit. The veins of the neck were distended. The heart was enlarged to the left, the apex lying in the fourth space, 11 cm. to the left of the midsternal line. A soft systolic murmur occurred over the apex; a louder systolic murmur was heard at the base and this was transmitted to the neck. The pulse rate was 104. A blood count on the day of admission gave 6,150 whites, with a mononuclear percentage of 29. The urine was normal.

An alimentary test for tolerance, 50 gm. of glucose, was made on Aug. 17, 1915, and 1.6 per cent. glycosuria obtained. Seventeen gm. of sugar were excreted and traces of sugar appeared in the urine for several days.

On Aug. 24, 1915, the upper poles of the thyroid were ligated.

The intravenous tolerance test was made on Feb. 3, 1916, with results as shown in Table 10. The weight of the patient was 53.2 kg.

TABLE 10.—GLUCOSE SOLUTION, 17.67 PER CENT. BY POLARISCOPE

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope	Total Gm.
0.5	2.51	31	0		
0.6	3.01	33	++	0.48	1.73
Subsequent loss.....					13.39
Total glycosuria.....					15.12

Tolerance lies between 0.5 and 0.6 gm. per kilogram per hour.

CASE 11.—Diagnosis, hyperthyroidism, myositis. J. S., aged 52, was admitted to the Presbyterian Hospital on the service of Dr. Frank Billings with complaints of soreness and weakness of the muscles. The trouble started acutely in May, 1915, with a fever of 104, headache, great muscle soreness and severe weakness. He lost 50 pounds in weight.

Examination revealed extensive muscular atrophies and contractures and tenderness of many tendons. There were no deformities of the joints. In addition, the thyroid was moderately enlarged and there was a fine tremor of the hands and tongue. Ocular symptoms were absent. There was also a diffuse dilatation of the heart and a loud mitral systolic murmur could be heard at the apex. Both great toe nails were ingrown and infected, the tonsils appeared diseased and the teeth were in bad condition. Blood counts gave 3,700,000 erythrocytes, 65 per cent. hemoglobin and 6,300 leukocytes, of which 42 per cent. were mononuclears. Urinalyses were normal. The pulse was fast, ranging from 90 to 120. The temperature proved irregular, occasionally mounting to 101 or 102 F.

TABLE 11.—GLUCOSE SOLUTION, 22.36 PER CENT. BY POLARISCOPE

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope	Total Gm.
0.5	1.75	44	0		
0.6	2.1	41	0		
0.7	2.45	46	+	0.21	0.34
Subsequent loss.....					1.94
Total glycosuria.....					1.97

The threshold of tolerance lies below 0.7 gm. per kilogram of body weight per hour.

From October 6 to October 12 the patient received 9 gm. of thyroid extract daily and within forty-eight hours the thyroid became more prominent and the nervousness and tremors increased. The thyroid extract was discontinued. The tonsils were removed and the teeth attended to.

An oral sugar tolerance test was made on October 22. One hundred gm. of glucose given on the fasting stomach produced no glycosuria.

An intravenous glucose tolerance test was made on November 29, and resulted as shown in Table 11. The weight of the patient was 47 kg.

CASE 12.—Diagnosis, hyperthyroidism. A. W., a woman, aged 30, suffered from goiter, dyspnea, and palpitation for four years. An attack of tonsillitis with fever immediately preceded the first appearance of these symptoms. Another severe attack of tonsillitis three years later was followed at once by nervousness, tachycardia and loss of weight.

The patient presented a moderate enlargement of the isthmus of the thyroid and a von Graefe. The pulse ranged from 90 to 110. Exophthalmos and tremor were absent. The tonsils were atrophic, but showed in their scarred fibrous surfaces and adhesion to the pillars definite evidence of infection. A moderate anemia was found. The leukocyte count was 8,000 and the mononucleosis 45 per cent. The urine was normal.

An intravenous tolerance test resulted as shown in Table 13. The weight of the patient was 49.54 kg.

TABLE 12.—GLUCOSE SOLUTION, 40.59 PER CENT. BY POLARISCOPE

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope	Total Gm.
0.5	1.02	60	0		
0.7	1.42	30	++	0.43	0.75
Subsequent loss.....					0.95
Total glycosuria.....					1.70

The threshold of tolerance lies well below 0.7 gm., but above 0.5 gm. per kilogram of body weight per hour.

CASE 13.—Diagnosis, myxedema. Mrs. A. L., aged 64, was admitted to the Presbyterian Hospital on Aug. 15, 1916, with complaints of thickened skin, stiffness of the hands and feet and mental dullness. The family history was negative except for one daughter, who developed an exophthalmic goiter when 40 years of age. The patient has had twelve healthy children, and gives no history of syphilis. She was always well up to her 61st year, when symptoms of the present trouble appeared.

The diagnosis of myxedema was made at the Central Free Dispensary in 1913, and for several months the patient received thyroid extract, but for the last two years she has been away and has had no treatment.

Examination showed the patient to be an elderly Jewish woman who appeared uninterested in her surroundings and mentally dull. The skin was dry and unstretched tight over the arms and legs. It had a board-like or bacony feel, especially over the face and arms. There were no axillary hairs, the eyebrows were scanty, and the hair of the scalp was thin and very dry. The pulse was slow, 60 to 70, and the respiration was shallow. The thyroid was not palpable. The lungs had normal resonance, and the breath sounds were clear, except for occasional moist râles at the base. The abdomen was negative.



The temperature was normal. The blood count gave 3,780,000 red cells, 8,100 white and a hemoglobin percentage of 75. The urine was normal in amount and free from albumin or sugar.

The intravenous tolerance test gave results as shown in Table 12. The weight of the patient was 70 kg.

TABLE 13.—GLUCOSE SOLUTION, 18.21 PER CENT. BY POLARISCOPE

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope	Total Gm.
0.8	5.13	48	0		
1	6.41	25	++	0.97	2.14

Subsequent loss not determined.

The second injection rate is well above the patient's tolerance, which undoubtedly lies between 0.8 and 0.9, the normal limits.

CASE 14.—Diagnosis, acromegaly. W. F., aged 40, was admitted to the service of Dr. Dean Lewis of the Presbyterian Hospital in 1913, with complaints of blindness and an increase in the growth of the hands, feet and head. His attention was first attracted to his condition in 1905, when he found that his hats were small. A year later his feet outgrew his shoes. In 1907 he was troubled with blurred vision and photophobia. His color sense was lost and finally in 1909 he became totally blind. His weight remained nearly stationary, about 225 pounds, during the entire course of the disease.

Examination revealed conditions typical of acromegaly, hypertrophied bones of the calvarium and jaw, huge hands and enormous feet. The skin was thick and the hair coarse and dry. An examination of the eyes revealed a complete amblyopia, due to a bilateral optic atrophy. There was also a marked horizontal nystagmus to the left. A Roentgen-ray plate of the skull showed an enormous sella.

The patient remained in the hospital until May, 1915. During this interval the temperature was practically normal and the pulse varied between 80 and 100. Twenty-four-hour urinary volumes were rather high, at times over 3,000 c.c. The urine often contained small amounts of albumin and a few casts, but never any sugar.

TABLE 14.—GLUCOSE SOLUTION, 19.43 PER CENT. BY POLARISCOPE

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope	Total Gm.
0.8	6.91	35	Trace	0.16	0.03
1	8.64	30	++	0.8	0.29
Subsequent loss.....					2.01
Total glycosuria.....					2.33

The threshold of tolerance lies very close to 0.8 gm. per kilogram of body weight per hour.

An oral glucose tolerance test was made on March 15, 1915. On giving 125 c.c. of glucose at 7 a. m., no glycosuria resulted.

The patient returned to the hospital in November. His condition had remained practically stationary. An intravenous tolerance test was made and resulted as shown in Table 14. The weight of the patient was 103.6 kg.

CASE 15.—Diagnosis, acromegaly. C. H., man aged 38 was admitted to the service of Dr. Dean Lewis of the Presbyterian Hospital on Jan. 4, 1916, with complaints of diminishing vision, increasing size, headache, weakness and loss of sexual desire. The patient was an adopted child and could tell nothing about his parents. At 7 years of age he had a severe illness the nature of which he ignores, and in 1906 suffered from a light attack of malaria. He was married in 1915 and had no children. The present illness seems to have begun in 1913, when he began to increase in size and weight. He is now 6 feet 1 inch tall and weighs 210 pounds. His former weight was 185. His feet and hands are bigger and he is obliged to buy  $7\frac{1}{4}$  inch size of hats instead of his former size 6 $\frac{3}{4}$ . Visual disturbance first occurred in February, 1915.

On examination the findings were those typical of acromegaly and roentgenograms of the hypophysial region revealed a sella very much enlarged. The leukocyte count was 7,600, with 58 per cent. mononuclears. The urine was normal on repeated examination. Two hundred gm. of glucose were given by mouth without producing glycosuria.

An intravenous tolerance test resulted as shown in Table 15. The weight of the patient was 95.5 kg.

TABLE 15.—GLUCOSE SOLUTION, 19.01 PER CENT.

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscopes	Total Gm.
0.7	5.85	30	0		
0.9	7.52	30	++	3.18	2.7
Subsequent loss.....					7.98
Total glycosuria.....					10.68

Tolerance lies below 0.9 gm., but above 0.7 gm. per kilogram per hour.

CASE 16.—Diagnosis, hypophysial disease (Frölich syndrome). H. C., a girl, aged 14, complained of obesity, delayed sexual development and frontal headache. The headaches began when she was about 10 years old. At 12 obesity was noticeable. At 13 she became uncertain of her balance and would sometimes fall to the ground, without, however, losing consciousness. She has never menstruated.

Examination revealed a corpulent child of small stature, mentally very bright. The head was larger than normal. The vision was good and the color fields were unaltered. The pupils, however, reacted sluggishly and both optic disks were abnormally pale, so that an early optic atrophy was diagnosed. Secondary sexual characteristics were lacking.

Urine examinations were completely negative and the twenty-four-hour urinary amounts were normal. The spinal fluid contained only 5 cells per c.mm. and gave negative Wassermann, Nonne and Lange tests. Roentgen-ray plates of the head showed a sella much increased in size. A subsequent decompression operation revealed marked intracranial pressure.

An intravenous glucose tolerance test was made on this patient before the decompression operation was performed and resulted as shown in Table 16. The weight of the patient was 47 kg.

TABLE 16.—GLUCOSE SOLUTION, 19.43 PER CENT. BY POLARISCOPE

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope	Total Gm.
0.8	8.28	42	+	0.81	0.872
Subsequent loss.....					0.405
Total glycosuria.....					0.777

The threshold of tolerance lies a little below 0.8 gm. per kilogram of body weight per hour. There is certainly no increase over normal.

CASE 17.—Diagnosis, hypophysial disease (gigantism?). T. W., a man, aged 26, was admitted to the service of Dr. D. P. Abbott at the Presbyterian Hospital on Jan. 10, 1916, with complaints of temporal headaches, weakness and nervousness. The headaches were of only a few days' duration. The weakness was not great. The nervousness had been present since childhood. The patient had no severe illnesses other than a chronic infection of the right ear which began early in childhood and only healed in 1912. He was, however, slow in learning to walk (2 years), was always big for his age and had always been backward in school. His family history was negative and venereal infection was denied.

Examination revealed a man 6 feet 1 inch tall, and big in proportion. The sexual development, pubic hair, etc., appeared normal. There was an old perforation in the right ear drum. A roentgenogram of the hypophysial region showed an unusually small sella and it was thought probable that a correlation existed between this and the rather peculiar development.

An intravenous glucose tolerance test resulted as shown in Table 17. The weight of the patient was 80.7 kg.

TABLE 17.—GLUCOSE SOLUTION, 19.2 PER CENT. BY POLARISCOPE

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope	Total Gm.
0.2	1.4	30	0		
0.7	4.9	30	0		
0.8	5.6	30	++	.....	Not determined

Tolerance lies between 0.7 and 0.8 gm. per kilogram per hour. It may be subnormal, but is certainly not increased.

Blood sugar determinations were made before and after the injections by Dr. G. Pearce and showed a percentage of 0.115 and 0.212, respectively.

CASE 18.—Diagnosis, hypophysial disease, diabetes insipidus. M. L., a man, aged 40, was admitted on the service of Dr. Joseph Miller at the Cook County Hospital in August, 1915. His complaints were polyuria and sexual impotence.



The first symptoms of disease occurred in 1907 and consisted of severe headache, occasional vomiting and excessive thirst. He would drink from 10 to 12 gallons of water a day. Sexual power was lost in 1911. Since 1913 he has been free from headaches and the thirst has been somewhat less. The appetite has always been moderate. He had four or five gonorrheal infections, and in 1901 a genital chancre. He was treated for this and no secondary symptoms of syphilis followed.

Examination revealed a moderately corpulent man of medium height and with small bones. The pubic hairs were found scanty and had a feminine distribution. The beard was very thin. Penis and testicles were small. In the right eye was a cataract, in the left the vision was good, but the red and green color fields showed some interlacing of their margins. The urine was found to be large in amount, the twenty-four-hour specimens containing from 3,000 to 6,000 c.c. The specific gravity was low and sugar was absent. Roentgenograms of the head revealed a slight enlargement of the sella and destruction of the posterior clinoid processes.

The intravenous glucose tolerance test resulted as shown in Table 18. The weight of the patient was 63.6 kg.

TABLE 18.—GLUCOSE SOLUTION, 20.11 PER CENT. BY POLARISCOPE

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope	Total Gm.
0.5	2.64	43	0		
0.6	3.16	50	0		
0.8	4.22	60	0		
0.9	4.75	87	+	0.29	0.59
Subsequent loss.....					0.86
Total glycosuria.....					1.45

The threshold of tolerance lies between 0.8 and 0.9 gm. per kilogram of body weight per hour.

CASE 19.—Diagnosis, cirrhosis of the liver. G. R., a man, aged 47, was admitted to the service of Dr. Billings at the Presbyterian Hospital on June 29, 1916. His complaints were loss of weight, weakness and pigmentation of the skin.

The history contained a record of scarlet fever at the age of 11 and malaria at 25, but excepting these illnesses, good health up to the spring of 1916.

Since April he has felt weak, has decreased in weight from 202 to 162 pounds, and has observed a pigmentation of the skin of the entire body. There has never been any icterus of the sclera and the color of the skin is more brown than yellow. In May he visited the dispensary, and from there was sent to the hospital. His family and venereal records are negative, but for years he has been drinking daily very considerable quantities of beer and whisky.

Examination revealed a poorly nourished man of average height. The pigmentation of the skin was unequally distributed, the nipples, pubic region and axillae were very dark; there were no spots in the mouth and the sclera were clear. There was a moderate pyorrhea and the teeth were discolored. The chest was normal in shape, the lungs slightly emphysematous, the heart borders somewhat wider than normal. A slight systolic blow was heard at the apex. The abdomen was somewhat pendulous, but contained no fluid. The spleen was palpable during inspiration, very firm, smooth and not tender.

The upper border of the liver was found at the fourth rib; the lower border extended 6 cm. below the costal arch in the mammary line. The edge was firm and regular and the surface was smooth.

The blood pressure, systolic, was 140, diastolic 70. Wassermann tests were negative. A blood count gave 3,500,000 erythrocytes, 4,000 leukocytes and 70 per cent. hemoglobin.

The urine was examined repeatedly without finding sugar or bile pigments. The stools contained bile in normal amount.

On July 2, 50 gm. of glucose were given on the empty stomach without leading to melituria. Levulose and galactose tolerance tests were omitted, owing to the impossibility of obtaining these sugars.

An intravenous d-glucose tolerance estimation, made on July 5, 1916, resulted as shown in Table 19. The weight of the patient was 74 kg.

TABLE 19.—GLUCOSE SOLUTION, 17.48 PER CENT. BY POLARISCOPE

Rate of Injection		Minutes of Injection	Urine Glucose	
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope
0.4	2.82	40	0	
0.6	4.28	20	0	
0.8	5.64	15	0	0

Tolerance is above 0.8 gm. per kilogram of body weight per hour and is apparently normal.

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## THE EFFECT OF BANDAGING OF THE LEGS ON THE RATE OF BLOOD FLOW IN THE FEET \*

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It has been suggested by some observers of the condition known as "trench-foot," which was so common among the soldiers in the trenches in Belgium and France, especially during the first winter of the war, that obstruction of the venous return by the puttees worn by the British troops was an important contributory factor.

The observations which form the subject of this paper are concerned only with the effect of relatively short applications of the puttees. Having been made in a laboratory on a normal man, they do not reproduce what is probably an important condition for the development of trench-foot, if the pressure of the puttees has anything to do with the condition, namely, the swelling under an already tight, wet, and dirty bandage. So far as the technic of the observations is concerned, it would have been easy enough to study the circulatory changes at first hand on soldiers. Not having been able to do this (and of course in such matters the military authorities must be the final judges as to what military exigencies will allow), the writer is, for the present at least, obliged to content himself with publishing a few specimen results on a normal man, which he had hoped to compare with data obtained by clinical studies of the actual condition at different stages. Since, however, so far as I am aware, no investigation of the influence of such bandages applied to the legs on the flow of blood in the feet has been published, and since such measurements have an interest in other relations, the application of bandages to limbs being so common in surgery and in certain medical procedures, it seems worth while to record the result of this preliminary study.

The observations were made on M. C., a healthy man, aged 26 years, weight 165 pounds, height 5 feet, 10 inches. Numerous measurements of the blood flow in his hands and feet have been made in the past few years, so that the range of variation of the flow under the

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\* From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University School of Medicine, Cleveland.



conditions of the observations is well known.<sup>1</sup> In all the experiments the amount of water in the calorimeters was 2,550 c.c. The puttees were always applied over the trousers, which were tucked in in the usual way.

October 22. M. C., four hours after breakfast, put his feet in the bath at 11:02 and in calorimeters at 11:18 a. m. The pulse was 90. At 11:40 a puttee was put on the right leg with the ordinary degree of firmness. At

TABLE 1

Time	Temperature of Calorimeters		Room Temperature	Time	Temperature of Calorimeters		Room Temperature
	Right	Left			Right	Left	
11:17	31.08	31.22		12:05	31.795	31.50	24.1
11:20	31.10	31.24	24.3	12:07	31.82	31.63	
11:22	31.24	31.27	24.3	12:09	31.85	31.65	24.1
11:24	31.28	31.29	24.3	12:11	31.88	31.675	
11:26	31.32	31.30		12:13	31.91	31.69	24.1
11:28	31.36	31.34	24.4	12:15	31.96	31.74	
11:30	31.395	31.36		12:17	32.00	31.775	24.1
11:32	31.41	31.37	24.2	12:19	32.06	31.83	
11:36	31.44	31.39	24.2	12:21	32.10	31.86	24.0
11:38	31.47	31.40	24.3	12:23	32.14	31.90	
11:40	31.495	31.43		12:25	32.19	31.94	24.1
11:43	31.52	31.44	24.2	12:27	32.23	31.97	
11:45	31.53	31.45		12:29	32.295	32.04	24.1
11:47	31.55	31.455	24.2	12:31	32.34	32.08	
11:49	31.58	31.46	24.2	12:33	32.39	32.125	24.1
11:51	31.60	31.465		12:36	32.43	32.16	
11:53	31.62	31.47	24.2	12:38	32.47	32.18	24.1
11:55	31.63	31.475		12:40	32.49	32.195	
11:57	31.66	31.485	24.2	12:42	32.50	32.22	24.1
11:59	31.68	31.50		12:44	32.52	32.24	
12:01	31.72	31.54	24.2	12:46	32.44	32.19	
12:03*	31.76	31.57		1:01	32.12	31.87	

\* Right foot feels more comfortable than left, and a little warmer.

12:23 the puttee was rapidly taken off. At 12:44 the feet were removed from the calorimeters.

The cooling of the calorimeters in fifteen minutes was 0.32 C. The volume of the right foot was 1,171 c.c., of the left 1,128 c.c. The water equivalent of the calorimeters with their contents was, right 3,616 c.c., left 3,584 c.c. The rectal temperature was 36.89 C.

In Table 1 is given an experiment in which a puttee was applied only to the right leg. Since the pressure is not the only effect which

1. Stewart, G. N.: Jour. Exper. Med., **18**, 354, 372.

the bandage can exert on the blood flow, but the warmth may also be important, in other experiments puttees were applied to both legs, but with different degrees of tightness.

In this experiment the flow in the feet was less than in the others cited, possibly because the subject had been going about for some time with bare feet and legs, completing the preparations for the observations; probably also in part because the experiment was made several hours after a meal. The taking of food, since it increases metabolism, must also increase the heat loss, and therefore the vasodilatation of the skin. The initial vasoconstriction due to exposure of the feet

TABLE 2

Time	Temperature of Calorimeters		Room Temperature	Time	Temperature of Calorimeters		Room Temperature
	Right	Left			Right	Left	
2:37	30.89	30.95		3:10	32.95	32.875	
2:40	30.99	31.03	24.0	3:12	33.01	32.95	24.0
2:42	31.20	31.20	24.1	3:14	33.09	33.03	24.1
2:44	31.40	31.39	24.2	3:16	33.18	33.13	
2:46	31.57	31.55	24.3	3:18	33.26	33.19	24.1
2:46	31.73	31.71	24.3	3:20	33.36	33.30	
2:50	31.89	31.86		3:22	33.45	33.37	24.1
2:52	32.02	32.99		3:24	33.53	33.46	
2:56	32.24	32.18	24.1	3:26	33.61	33.55	24.1
2:58	32.33	32.28	24.1	3:28	33.70	33.65	
3:00	32.45	32.38		3:30	33.78	33.72	24.15
3:02	32.58	32.51	24.0	3:32	33.86	33.785	
3:04	32.72	32.65		3:34	33.81	33.74	
3:06	32.80	32.74	24.0	3:46	33.50	33.43	
3:08	32.88	32.80	24.0				

tended to pass off as the experiment proceeded, the rate of flow increasing in the successive periods. But there was no indication that the puttee on the right leg in any way interfered with the flow in the right foot. The ratio of the minute flow in the left foot to that in the right was almost precisely the same in the eighteen minutes before the application of the puttee as in the forty minutes during which it remained on. The data are displayed in succinct form in Table 5.

In the experiment shown in Table 2 a puttee was applied firmly to the right leg. The degree of pressure of the puttee was about what the soldier commonly employs. On the left leg a similar puttee was applied quite loosely, so that the heat-conserving effect would be the

same as on the right leg, while the pressure was negligible. For the ten minutes before the application of the puttees the flow per minute per 100 c.c. of part was 7.18 gm. for the right and 7.2 gm. for the left foot, practical equality. For twenty minutes with the puttees on, the flow was 6.43 gm. and 6.58 gm. per minute per 100 c.c. For fourteen minutes after removal of the puttees the flow was 8.09 gm. and 8.13 gm. per 100 c.c. per minute for the right and left foot respectively.

TABLE 3

Time	Temperature of Calorimeters		Room Temperature	Time	Temperature of Calorimeters		Room Temperature
	Right	Left			Right	Left	
2:36	30.87	30.88		3:17	33.33	33.22	24.2
2:38	30.93	30.97	24.1	3:19	33.40	33.27	
2:40	31.09	31.09	24.1	3:21	33.47	33.34	24.2
2:42	31.23	31.27		3:23	33.52	33.42	
2:44	31.42	31.43	24.1	3:25	33.595	33.47	24.2*
2:46	31.53	31.55		3:27	33.65	33.54	24.25
2:48	31.70	31.68	24.0	3:29	33.72	33.59	
2:50	31.85	31.82		3:31	33.78	33.66	24.2
2:52	31.99	31.92	24.1	3:33	33.83	33.72	
2:54	32.12	32.055		3:35	33.90	33.79	24.2
2:56	32.25	32.16	24.1	3:37	33.97	33.86	
3:00	32.41	32.34	24.3	3:39	34.02	33.915	24.2
3:02	32.53	32.45	24.25	3:41	34.07	33.96	
3:05	32.70	32.60	24.3	3:43	34.13	34.02	24.2
3:07	32.805	32.69		3:45	34.19	34.07	
3:09	32.90	32.80	24.25	3:47	34.26	34.14	24.2
3:11	33.00	32.91		3:49	34.30	34.18	
3:13	33.11	33.03	24.2	3:51	34.26	34.12	
3:15	33.22	33.13		4:05	33.90	33.76	

\* Left leg felt cool after the puttee was taken off it; the right leg felt warm.

October 26. M. C., had lunch one hour before, and had walked outside for some time. The pulse was 94. The feet were put in the bath at 2:22, and in calorimeters at 2:39 p. m. At 2:52 a puttee was put firmly on the right leg and one slackly on the left leg. At 3:16 the puttees were taken off quickly. At 3:32 the feet were taken out of the calorimeters.

The cooling of the calorimeters in twelve minutes was 0.31 C. The volume of the right foot was 1,180 c.c., of the left 1,128 c.c. The water equivalent of the calorimeters with their contents was, right 3,623 c.c., left 3,385 c.c. The rectal temperature was 37.05 C.

If the twenty minute period is analyzed, it is seen that for the first half of it the flow was 6.84 gm. and 6.97 gm. for the two feet,



respectively; and for the second half 6.13 gm. and 6.3 gm. The diminution in the right foot is not due to pressure of the bandage, since it is the same as the diminution in the left. Removal of the puttees was followed by some increase in flow in both feet, but without disturbance of the relation of equality in the two feet present from the beginning of the experiment. The increased flow cannot be interpreted as due to the vasomotor paralysis following the removal of a tight bandage described by Bier. For the puttee on the right leg was not so tight as to occasion the slightest discomfort, and the increased flow was present in the left foot as well as in the right.

TABLE 4

Time	Temperature of Calorimeters		Room Temperature	Time	Temperature of Calorimeters		Room Temperature
	Right	Left			Right	Left	
11:26	30.92	30.78		12:05	32.46	31.99	24.15
11:29	31.00	30.86	25.2	12:07*	32.56	32.08	24.2
11:31	31.07	30.92	25.2	12:09	32.61	32.14	24.2
11:33	31.17	30.98	25.2	12:11	32.68	32.18	
11:35	31.27	31.07	25.1	12:13	32.73	32.23	24.2
11:37	31.37	31.16	24.7	12:15	32.80	32.28	24.25
11:39	31.49	31.26	24.4	12:17	32.88	32.34	
11:41	31.59	31.34		12:19	32.96	32.40	24.2
11:45	31.71	31.43	24.5	12:21	33.00	32.455	
11:47	31.76	31.45	24.4	12:23	33.07	32.51	24.2
11:49	31.81	31.48	24.3	12:25	33.09	32.55	
11:51	31.895	31.55		12:27	33.13	32.59	24.1
11:53	31.96	31.59	24.1	12:29	33.17	32.64	24.1
11:55	32.04	31.65	24.0	12:31	33.19	32.66	
11:57	32.16	31.755	24.0	12:33	33.20	32.68	24.0
11:59	32.22	31.81	24.0	12:35	33.20	32.68	
12:01	32.28	31.85	24.05	12:37	33.12	32.615	
12:03	32.37	31.91	24.1	12:49	32.81	32.31	

\* The right leg was comfortable, the left somewhat tired and uncomfortable.

In the experiment shown in Table 3 the procedure was the same as in the last experiment, except that the puttee on the left leg (put on quite slack) was removed some time before the other, in order to see whether the increased loss of heat from the left leg caused any effect on the flow in the right foot. There was, as a matter of fact, a slight diminution in the flow in the left foot, which might be attributed to vasoconstriction due to increased cooling. If this was the cause,

the action extended reflexly to the right limb, the puttee on which had not been disturbed, as the ratio of the flows in the two feet remained unchanged. There was no indication that the pressure of the puttee on the right leg diminished the flow in the right foot.

October 29. M. C., one hour after lunch, put his feet in the bath at 2:20, in the calorimeters at 2:37 p. m. The pulse was 88. At 2:56 a puttee was

TABLE 5

Date	Pulse Rate	Temperature (C.) of				Volume of Foot in C.c.		Heat Given Off in Gm.—Calories		
		Room	Arterial Blood	Calorimeters		Right	Left	Right	Left	No. M
				Right	Left					
10/22	90	24.3	36.29	31.37	31.35	1,171	1,128	2,115	1,792	18
		24.1	.....	21.82	31.67	.....	.....	5,170	4,551	40
		24.1	.....	32.36	32.09	.....	.....	2,680	2,530	19
10/26	94	24.2	36.45	31.61	31.60	1,180	1,128	3,695	3,549	10
		24.05	.....	32.52	32.46	.....	.....	2,855	2,825	10
		24.05	.....	32.99	32.94	.....	.....	2,253	2,248	10
		24.1	.....	33.56	33.49	.....	.....	3,478	3,423	14
10/29	88	24.1	36.36	31.92	31.86	1,190	1,133	3,158	2,913	10
		24.25	.....	32.87	32.78	.....	.....	4,720	4,520	17
		24.2	.....	33.56	33.43	.....	.....	2,051	2,027	10
		24.2	.....	34.04	33.92	.....	.....	3,557	3,516	18
11/ 2	80	24.8	36.34	31.38	31.16	1,171	1,142	2,133	1,905	8
		24.3	.....	31.88	31.54	.....	.....	2,007	1,600	10
		24.1	.....	32.36	31.92	.....	.....	3,743	3,290	16
		24.2	.....	32.90	32.37	.....	.....	2,155	1,923	10
		24.1	.....	33.14	32.60	.....	.....	1,610	1,725	12
11/30	100	20.5	36.41	31.19	31.05	1,171	1,142	6,183	4,602	20
		20.6	.....	31.49	31.23	.....	.....	3,073	2,394	10
		20.9	.....	32.19	31.74	.....	.....	2,759	2,459	10
		21.1	.....	32.60	32.10	.....	.....	1,797	1,675	6
		21.2	.....	33.13	32.57	.....	.....	3,952	3,703	18
		21.0	.....	33.53	32.92	.....	.....	1,121	1,064	6

put firmly on the right leg and one slackly on the left leg. At 3:17 the puttee was rapidly taken off the left leg; at 3:29 off the right leg. The feet were taken out of the calorimeters at 3:49.

The cooling of the calorimeters in fourteen minutes was 0.36 C. The volume of the right foot was 1,190 c.c., of the left 1,133 c.c. The water equivalent of the calorimeters with their contents was, right 3,630 c., left 3,588 c.c. The rectal temperature was 36.96 C.

Indeed, with both puttees on, the flow was slightly and equally increased in both feet, probably on account of the heating effect.

In the next experiment to be quoted (Table 4) the puttee was purposely put on the left leg so tightly as to produce a certain amount of discomfort, while it was applied to the right leg with the ordinary, comfortable degree of pressure. The subject was hungry (four and

TABLE 5—(Continued)

Blood Flow in Gm. per Min.		Flow per 100 C.c. of Foot per Min.		Ratio of Flow in 2 Feet	Remarks
Right	Left	Right	Left		
26.68	22.39	2.26	1.98	1:1.14	Before puttee was put on
32.20	27.37	2.75	2.42	1:1.14	Puttee on right leg
39.88	35.23	3.40	3.12	1:1.09	Puttee off
84.82	81.31	7.18	7.20	1:1.00	Before puttees put on; after eating
80.72	78.67	6.84	6.97	1:1.02	Put. R. firm, L. slack (first 10 minutes)
72.35	71.16	6.13	6.30	1:1.03	Put. R. firm, L. slack (next 10 minutes)
95.51	91.78	8.09	8.13	1:1.00	Puttees off
79.03	71.92	6.64	6.34	1:1.05	Before puttees put on; after eating
88.39	82.52	7.42	7.28	1:1.02	Put. R. firm, L. slack
81.39	76.87	6.88	6.78	1:1.01	Puttee off L. leg
94.64	88.94	7.95	7.85	1:1.01	Puttees off both legs
59.73	51.08	5.10	4.47	1:1.14	Before puttees; after eating
50.00	37.03	4.27	3.24	1:1.31	Put. R. firm, L. too tight (first 10 minutes)
65.31	51.69	5.57	4.52	1:1.23	Put. R. firm, L. too tight (next 16 minutes)
69.60	53.82	5.94	4.71	1:1.26	First 10 minutes after puttees removed
46.58	42.70	3.97	3.73	1:1.06	Next 12 minutes after puttees removed
65.90	47.69	5.62	4.17	1:1.34	Before puttees put on
69.39	51.35	5.92	4.49	1:1.31	Last 10 minutes before puttees
72.64	58.51	6.20	5.12	1:1.21	First 10 minutes with puttees
87.34	71.97	7.46	6.80	1:1.18	Next 6 minutes with puttees
74.37	59.52	6.85	5.21	1:1.21	After tightening tapes on R. leg
54.06	42.34	4.61	3.70	1:1.24	After puttees off

one-half hours after breakfast) and the flow in the feet was less than in the experiments shown in Tables 2 and 3. The flow in the two feet was 5.10 gm. and 4.47 gm., respectively, per 100 c.c. per minute for an eight minute period before the application of the bandages (ratio of left to right 1 to 1.14). For a period of twenty-six minutes with the puttees on the flow was 5.13 gm. and 4.04 gm., respectively



(ratio 1 to 1.27). The cutting down of the flow by the improperly applied puttee on the left leg is evident, although the bandage was not put on so tight as to cause an extreme degree of discomfort.

November 2. M. C., four hours after breakfast, put his feet in the bath at 11:07, in the calorimeters at 11:27:30 a. m. At 11:41 puttees were put firmly on both legs, but the left was much tighter and less comfortable than the right. At 12:11 the puttees were rapidly taken off. The feet were taken out of the calorimeters at 12:35.

The cooling of the calorimeters in twelve minutes was, right 0.31 C., left 0.305 C. The volume of the right foot was 1,171 c.c., of the left 1,142 c.c. The water equivalent of the calorimeters with their contents was, right 3,616 c.c., left 3,595 c.c. The pulse was 80. Rectal temperature was 36.96 C.

If the period is analyzed, the deficit in the left foot is seen to be greatest in the first ten minutes (flow in right foot 4.27 gm. per 100 c.c. per minute, in left 3.24 gm., giving a ratio of 1 to 1.31). The flow in the right foot is also diminished somewhat. In the remaining sixteen minutes of the period of application of the puttees the flow increases in both feet, but relatively more in the left, as the venous pressure rises and forces the block (5.57 gm. for right and 4.52 gm. for left foot, ratio 1 to 1.23). Even with the tight bandage on the left leg, the initial flow in the left foot is soon reestablished. This, however, is in a healthy man, whose cutaneous flow is uniformly good. It seems clear enough that in men who habitually suffer from cold feet, and whose foot flow is therefore normally small, the injudicious application of a puttee might easily cause a harmful reduction in the flow which would render the foot more readily susceptible to the effects of cold and wet, of slight injuries and of the passive congestion associated with standing for long periods in one position. On the other hand, so far as can be judged from experiments of such relatively short duration, a properly adjusted puttee not only causes, under normal conditions, no permanent diminution in the foot flow, but may even somewhat increase the flow, probably largely because of its heat conserving property.

In another experiment (that of November 30) different methods of fastening the puttees were investigated. That on the right leg was tied by tapes an inch wide, that on the left leg was fastened smoothly by a safety pin. The tapes caused a somewhat tighter feeling, according to the subject, than the pin, but both legs felt comfortable. After the first sixteen minutes the tapes were made considerably tighter on the right leg, but not uncomfortably so. As will be seen in Table 5, the tightening of the tapes did not produce any diminution in the flow in the right foot as compared with that in the left, although the flow in both was now diminishing, and diminished still more after removal of the puttees. This last decrease in the flow, seen also in the experiment of November 2, may be due to a vasoconstriction associated with

cooling of the limbs after removal of the bandages. However, a terminal vasoconstriction due to the exposure is not uncommonly seen in long experiments on the foot flow when no bandages have been applied.

#### SUMMARY

It is shown that a puttee applied with the usual degree of pressure on the leg causes no diminution in the blood flow through the foot. When it is put on so tight that some discomfort is produced the flow in the foot is at first reduced.

## THE BILE CONTENT OF THE BLOOD IN PER- NICIOUS ANEMIA \*

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The lemon yellow color of the skin in pernicious anemia has always been closely associated with the idea of jaundice and is often referred to as an icteroid hue. But the almost invariable absence of bile from the urine has stood in the way of our calling pernicious anemia patients definitely jaundiced. The frequent appearance of urobilin in the urine has suggested an explanation for the color of the skin, and we frequently see the term "urobilin icterus" in this connection.

Hemolysis, which is the most definite pathologic entity of pernicious anemia, is also fundamentally associated with the symptom jaundice and we are therefore enticed by the term "hemolytic jaundice" in pernicious anemia.

Just what relation exists between the appearance of bile pigments in the blood and the yellow pigmentation of the skin in pernicious anemia has been the object of this investigation.

The presence of bile in the blood in any condition, physiologic or pathologic, has not been extensively investigated, and satisfactory methods have not been available. Rather extensive chemical studies of the blood in pernicious anemia have been made, especially by Erben,<sup>1</sup> Rumpf<sup>2</sup> and von Jakchs,<sup>3</sup> but these and others make no mention of biliary elements save that the amounts of cholesterin and lecithin are diminished; a finding that is common to many chronic diseases, especially those in which starvation is a factor. Sylaba<sup>4</sup> found bilirubin in the blood in eight cases of pernicious anemia. Wilbur and Addis,<sup>5</sup> in their observations on urobilin in the plasma, did not find that substance present in pernicious anemia.

### METHODS

Oxalated plasma was used in every case. Gmelin's test for bilirubin was used in testing for bile pigment, which gives the well-known blue-green color with nitric acid. This method was applied to the

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\* From the Medical Clinic of Lakeside Hospital.

1. Erben: *Ztschr. f. klin. Med.*, 1900, **40**, 266.

2. Rumpf: *Berl. klin. Wchnschr.*, 1901, **38**, 477.

3. Von Jakchs: *Klinische Pathologie des Blutes*, Jena, 1896, p. 311.

4. Sylaba: *Abst. in Folia Hematol.*, 1904, **1**, 283, 589.

5. Wilbur and Addis: *THE ARCHIVES INT. MED.*, 1914, **13**, 235.



plasma by Gilbert,<sup>6</sup> who arrived at conclusions as to the normal bilirubin content of the human plasma. Gmelin's test on the plasma is made by putting nitric acid under the plasma in a test tube, with a small pipet. A white coagulum is formed at once at the junction, which soon develops into a white layer of a certain thickness, and remains fairly constant as the acid dissolves the coagulum at the lower border and forms it at the upper, thus ascending through the plasma. The blue-green color develops in a line at the midst of the white zone and remains in the same relative position as long as there is any white zone. When bilirubin is present in small amounts, the blue color may be as long as a half hour in appearing. Since the white coagulum forms a good background on which to see the blue line, the tests are very satisfactory and give unequivocal results.

In a later series of observations, part of which is included in this report, a few specimens were examined for bilirubin by the Huppert-Cole method and out of twenty giving a positive Gmelin test all save one gave a positive Huppert-Cole in addition. The exceptional case, however, gave but a very faint Gmelin test. Included in this series of twenty are eight cases of pernicious anemia, six of which gave a positive Huppert-Cole test as well as a positive Gmelin, the remaining two being negative by both methods. It is the impression of the writer that the Huppert-Cole test is not so delicate as Gmelin's test.

Just what minimum amount of bilirubin gives Gmelin's test is not definitely known, but ordinary gallbladder bile can be diluted 600 times in colorless plasma and still give this test. Gallbladder bile contains about 2 per cent. pigment, chiefly bilirubin, so approximately the test will show one part in 30,000. But it is quite certain that a plasma may be visibly bile stained and still not give Gmelin's test, for ordinary gallbladder bile can be diluted 5,000 times and a yellow color be just perceptible in a column 1 cm. deep, and since only 2 per cent. of bile is bilirubin, we conclude that bilirubin can be diluted 250,000 times and still be just visible, making inspection of the plasma a test much more sensitive than Gmelin's.

There is no way of concentrating small amounts of pigment, nor is there any very satisfactory quantitative method for measuring bilirubin. The writer has accepted the staining of the plasma as a measure of bilirubin content, for no other yellow substance was found in any plasmas, and the intensity of Gmelin's test is proportional to the degree of yellow staining.

Lutein is said to stain the plasma yellow, as well as to give a blue-green color with nitric acid. Lutein can be differentiated from bilirubin in that it is not separated from its solution in chloroform by

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6. Gilbert: *Compt. rend. Soc. de Biol.*, 1905, 1906.

water containing an alkali (Hammarsten). A characteristic spectrum is also described for it. We have never been able to identify luetin in the plasma in any instance, in particular in one faintly stained plasma which gave a positive Gmelin's test, but not the Huppert-Cole test.

For the purpose of recording, the bilirubin content of the blood is expressed by the dilution required to diminish the staining to a point where it is just perceptible in a column 1 cm. deep. Plasma to be thus measured must be obtained free from hemolysis. In diluting the plasma with distilled water, a faint precipitate of globulin usually appears in suspension, which can be put in solution by a drop of ammonium hydroxid. The end point of the dilution, that is, where the yellow color is just perceptible, is found by comparing the solution in a small test tube with a similar column of distilled water. To facilitate reading, the tubes are held parallel and observation made down the length of the tubes, and a device which serves to bring the two columns to still more equal terms is to immerse the ends of the tubes in an inch or two of water in an evaporating dish. This cuts out all reflecting surfaces and gives a white background. While this by no means serves to make quantitative estimates of bilirubin in the blood, it does give a method for comparing and recording the jaundice of the plasma within about a 10 per cent. error. Specimens of plasma examined by this method showed a staining that could be diluted as high as 275 times. The lesser degrees of staining are not so satisfactorily estimated and no measurement under ten is very reliable. All at twenty or over gave a positive Gmelin's test.

Quite recently there has appeared a method<sup>7</sup> for measuring bilirubin in plasma, but as yet no general use has been made of it.

For urobilin Ehrlich's aldehyde test was employed on oxalate plasma, applied directly. Most of the blood protein is first precipitated by one or two volumes of saturated alcoholic solution of zinc acetate and centrifugalizing; 5 to 10 drops of the aldehyde reagent are added and a color from pink to dark red develops, which gives the characteristic spectrum if urobilin or urobilinogen is present. While negative results by this method are not always acceptable the writer has found the test positive in three instances and then strongly positive.

For detection of bile salts the Pettenkofer test was employed. The Pettenkofer test is not specific for bile salts or for any group and therefore all its results must be carefully criticised. On account of its lack of specificity the Pettenkofer reaction appears very seldom in the literature (other than biochemical). Pettenkofer, in his original description, named forty substances that give it. Of those that occur in the blood and are known to give it are cholesterin and lecithin;

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7. Hooper and Whipple: *Am. Jour. Physiol.*, 1916, **40**, 332.



also many alcohols, fatty acids and aromatic substances. The absorption spectrum of the Pettenkofer test is said to be more nearly specific than the color test alone, so we have controlled all our color tests by the spectroscopic examination in addition.

The Pettenkofer test cannot be applied directly to the blood or blood plasma, but the bile salts must be separated from the blood proteins and be relatively free from lecithin and cholesterin. This has been done by precipitating the salts with basic lead acetate and ammonium, after the proteins have been removed with large amounts of alcohol—the Hoppe-Seyler method—a process which is long and tedious, requiring much time and many manipulations. But it was found that the bile salts can be obtained relatively free by dialyzing the plasma through collodion sacs into water or water and alcohol.

This method has been employed in all cases. Five c.c. of plasma are dialyzed into from 5 to 15 c.c. of water or equal parts water and alcohol, and two or three such dialyzers are allowed to stand over night. The collected dialysate is then concentrated and the Pettenkofer test applied, and the spectroscopic examination made, if any color develops. For the test 2 c.c. of concentrated dialysate is placed in a small flask with 2 or 3 drops of a 1 to 1,000 aqueous solution, of furfurol, and 2 c.c. of concentrated sulphuric acid is added drop by drop from a pipet; but the contents of the flask are kept at 60 C. by immersing the flask in a bath at 60 C. and keeping the mixture agitated. When the acid is added too rapidly a dark, reddish-brown color sometimes appears from the charring action of the acid on the small amounts of protein or sugar that dialyze. If the temperature remains much below 60 C., the color fails to develop or appears very faint.

The writer is aware that by the above method the differentiation between bile salts and cholesterin is the most uncertain point of the process, since it depends on the difference in diffusibility of the two substances through collodion sacs. It is quite certain that bile salts dialyze very rapidly from specimens of bile obtained at operation, or from fistulae after operation; but cholesterin has not been found to dialyze.

Furthermore, when a dialysate from a plasma is evaporated to dryness and extracted with ether or chloroform, the substance in this case which gives the positive Pettenkofer is left behind. Also, bile salts have been recovered from several plasmas by the Hoppe-Seyler method, and by dialysis as well, where cholesterin, as estimated by the method of Weston and Kent, was not increased. But the writer has not acquired proficiency in this cholesterin method, and draws no definite conclusions as yet on this particular point.

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8. Weston and Kent: Jour. Med. Research, 1912, **26**, 531.



The spectrum of a Pettenkofer test for bile salts, obtained from human gallbladder bile and fistula bile by dialysis and by the Hoppe-Seyler method, shows a wide absorption band in the blue when the test is first made and is of a cherry red color. Later, as the cherry turns to purple, the band in the blue fades and a smaller band develops in the orange (near *D*, between *D* and *C*). In a strong reaction both may be visible at once, but in weaker reactions the band in the blue usually fades before the other appears. This same spectrum is given by bile salts dialyzed from the plasma, as well as by those precipitated by the Hoppe-Seyler method. Numerous plasmas dialyze salts in water that give the Pettenkofer test; that is, the cherry red or pink color turning to purple, but not in concentration sufficient to allow the positive identification of the absorption bands. So we have been obliged to accept as positive in many instances reactions that give the colors, but not the spectra.

It is not claimed in this report that every specimen which gave a positive Pettenkofer test was proved absolutely to contain bile salts. But it may be said, further, that the method stated gives positive reactions in many cases of jaundice in which bile salts appear in the urine and gives negative tests in those same cases after the bile salts have disappeared from the urine. It also seldom gives positive tests in normal plasma.

The Pettenkofer reaction was not found to be adaptable to quantitative measurements either colorimetrically or spectroscopically, but there is a distinct variability in the intensity of the color reaction as well as in the definition of the absorption bands. This is sufficiently clear, that these tests can be classified as plus, double plus and triple plus.

By these methods the writer has examined the plasma of twenty patients in whom a diagnosis of pernicious anemia had been made. That is, he has tried Gmelin's reaction; measured the staining of the plasma; tested for urobilin; dialyzed and examined the dialysate for bilirubin and tested it for bile salts with the Pettenkofer and spectroscopic tests. The results of this investigation are summarized in Table 1. In this table also are certain other observations that are immediately concerned, namely, the blood findings relative to the anemia, the skin jaundice, the biliary elements in the urine, and the urobilin in the stool. On the urine the Hammersten-Salkowski test was used for the bile pigments, Hay's test for the bile salts, and Ehrlich's aldehyde test for urobilin. For the stool Wilbur and Addis' method of measuring the total daily urobilin excretion was used. And to correlate with the clinical side the table is made to include an index of the two most significant symptoms in the cases studied, that is, the loss of strength and the nerve disturbances. In the latter the determination of ataxias

is made by the plan demonstrated by Hoover<sup>9</sup> in 1914; acro-ataxia being an ataxia of the fingers and toes; proximo-ataxia, an ataxia of the iliofemoral or of the thoracohumeral group of muscles.

#### RESULTS

**Bilirubin:** In brief, the table shows that out of the twenty cases, in sixteen there was found bilirubin in amounts sufficient to give a positive Gmelin test. In two of the remaining four the plasma was visibly jaundiced, but not deeply enough to give a test. The bilirubin showed a great variability, the figures ranging from 10 to 150. Of these, five specimens may be considered as containing bile above the threshold of the kidney, the threshold being established as 60 by similar observations on a group of cases of obstructive jaundice. However, bilirubin was found in the urine of none, regardless of the amount in the blood. Of the eighteen cases showing staining of the plasma, nine were visibly jaundiced in the sclerae; of the remaining nine, only one gave a history of ever being yellow.

Pigment dialyzed through the collodion sacs in but three cases. In these it was noticed on concentrating the dialysates that an unusually large amount of protein had dialyzed. In only one instance did sufficient pigment dialyze to allow its identification as bilirubin.

The cases showing the highest concentration of bilirubin showed the most marked evidence of rapid blood destruction, namely, increased output of urobilin in stool and urine, loss of strength from anemia, also the highest color index.

**Urobilin:** This was not found in the plasma of any, although it occurred in the urine of all save one, this one being a case which showed absolutely no staining of the plasma.

**Salts:** A positive Pettenkofer test was found in sixteen out of twenty plasmas. Of the four which did not give a positive Pettenkofer test, there were two plasmas which contained a large amount of bilirubin and two which contained none at all. There was no direct correspondence between the presence of salts or the amount of salts and the amount of bilirubin in the plasma. One plasma, with a jaundice of 75, gave a doubtful Pettenkofer, while two others with absolutely no staining of the plasma gave a strong Pettenkofer, and bile salts were found in the urine of none of the cases. There was no relation between the amount of bile salts and the blood destruction or the color index.

It was observed, however, that the cases giving the strongest Pettenkofer, hence highest concentration of salts, showed the most marked nerve lesions; in fact in three such cases the nerve lesions caused all the symptoms of the disease.

9. Hoover: *Am. Jour. Med. Sc.*, 1915, **150**, 651.

By these same methods the writer has examined the plasma of a series of patients selected on account of suspected liver disease, infections, anemias, etc., as well as many normals. Table 2 summarizes ten of these cases, nine being secondary anemias from various causes, and one being a case of Banti's disease. Of the nine secondary anemia

TABLE 1.—SUMMARY OF FINDINGS—

Name	Age, Years	Red Blood Cells	Hemo-globin, Tallquist, %	Color Index	Jaundice	History of Jaundice	Blood		
							Jaundice of Plasma	Gmelin's Test	Urobilin
L. H.	60	1,032,000	30	1.5	+	+	20	+	...
*		3,500,000	70	1	+	+	15	0	...
M. C.	52	1,200,000	40	1.7	+	+	60	+	0
B. S.	63	3,300,000	70	1.05	+	+	...	+	...
†		3,300,000	70	1.05	+	+	15	0	...
‡	64	3,400,000	70	1	0	+	15	0	0
S. H.	57	2,277,000	60	1.3	0	0	20	+	...
U. A.	52	2,312,000	70	1.5	0	0	0	0	...
F. C.	64	1,400,000	40	1.4	+	+	150	+	0
M. B.	48	3,324,000	80	1.05	0	0	15	0	...
L. C.	42	3,400,000	70	1	0	0	75	+	0
E. B.	39	1,206,000	70	2.9	0	0	50	+	0
J. W.	56	1,168,000	40	1.7	+	0	70	+	0
§		1,944,000	65	1.7	+	+	20	0	0
L. Y.	45	3,734,000	75	1	0	0	50	+	0
C. A.	60	2,256,000	75	1.7	+	0	50	+	...
#		1,200,000	45	2	+	+	25	+	0
H. K.	47	3,370,000	85	1.1	+	0	0	0	0
G. L.	42	574,000	40	1.8	+	+	70	+	0
F. F.	43	1,200,000	40	2	0	0	40	+	0
J. L.	56	3,132,000	80	1.3	0	0	25	+	0
M. H.	27	960,000	30	1.5	0	0	35	+	0
V. B.	39	2,656,000	65	1.5	0	0	35	+	0
B. P.	67	1,848,999	50	1.4	0	0	10	0	0
C. Z.	39	1,024,000	45	2.1	0	+	25	+	0

\* L. H. three months later.

† B. S. three months later.

‡ B. S. one year later.

§ J. W. two months later.

# C. A. nine months later.

cases, none showed any staining of the plasma, although two of the patients were described by the ward physician as appearing jaundiced at the time the specimens were taken. Two of the secondary anemia cases had bile salts in the plasma. The patient with Banti's disease was jaundiced and had both bile pigments and salts in the plasma, but neither in the urine.



In a group of about forty cases, selected on account of liver disease, principally alcoholic and infectious cirrhosis, disease of the bile ducts, general infections with jaundice, and jaundice after transfusion, bile pigment and bile salts were found in the plasma in varying amounts, and in varying relations to each other and to choluria. The interpre-

## —IN PERNICIOUS ANEMIAS

Dialysis		Urine			Stool Urobilin	Loss of Strength	Neurologic Symptoms			
Bile Pigment	Bile Salts	Bile Pigment	Bile Salts	Urobilin			Vibration Test	Aero-paras-thesias	Aero-Ataxia	Prox-imo-Ataxia
0	0	0	0	+	Increased.....	Marked.....	0	+	+	0
0	+	0	0	0	.....	Less marked..	0	0	0	0
0	+	0	0	+	Increased.....	Very great....	+	+	0	0
0	+	0	0	+	.....	Marked.....	+	+	+	0
0	+	0	0	0	.....	Marked.....	+	+	+	0
0	++	0	0	0	.....	Marked.....	+	+	+	0
0	+	0	0	+	Increased.....	Marked.....	+	+	+	+
0	++	0	0	+	.....	Slight.....	+	+	+	+
?	+	0	0	+	Much increased..	Great.....	0	0	+	+
0	0	0	0	+	Slight increase...	None.....	+	+	0	0
0	?	0	0	+	.....	None.....	+	+	+	0
0	?	0	0	+	Increased.....	Very great....	0	+	0	0
0	+	0	0	+	Much increased..	Very great....	+	+	+	0
0	+	0	0	0	Increased.....	Slight.....	+	+	+	0
0	+	0	0	+	.....	Slight.....	0	0	0	0
0	+	0	0	+	.....	Slight.....	+	+	+	+
0	+	0	0	0	.....	Slight.....	+	+	+	+
0	++	0	0	0	.....	Very great....	+	+	+	+
0	+	0	0	+	Increased.....	Marked.....	+	+	+	0
+	+	0	0	+	.....	Great.....	0	+	+	0
0	+	0	0	+	Increased.....	Slight.....	+	+	+	0
+	+	0	0	+	Increased.....	Very great....	0	0	0	0
+	+	0	0	+	Increased.....	Slight.....	0	0	0	0
0	0	0	0	+	.....	Marked.....	+	+	+	+
0	+	0	0	+	Increased.....	Marked.....	+	+	+	0

tation of the blood jaundice in the various conditions affords interesting speculation, but much remains to be done before any conclusions are justified. A few main points are clear, namely, that jaundice of the sclera or skin is of course always associated with jaundice of the plasma, but the converse is not true. We have frequently been surprised by obtaining a distinctly jaundiced plasma from a patient who had no suggestion of jaundice of the skin. Also, the presence of bile

salts in the blood is associated with some disease of the liver, which is in accord with the idea most commonly held, that bile salts are formed only in the liver.

On the basis of experience with jaundice of the plasma in the various diseases of the liver, and the frequent appearance of bile salts in the blood in pernicious anemia, one interpretation of our findings in the cases tabulated can be ventured, namely, that the jaundice is hepatic in origin. From the fact that bile pigment in all these cases was not found in the urine, and, furthermore, usually does not dialyze through collodion membranes into water, it is assumed that the pigment is fixed to the plasma, perhaps chemically. This fixation does not seem to depend on the intensity or duration of the jaundice. Why the bile salts are diffusible from the plasma through collodion membranes and do not appear in the urine we have no theory.

TABLE 2.—SUMMARY OF FINDINGS IN BLOOD—

Name	Age, Years	Diagnosis	Red Blood Cells	Hemo- globin, per Cent.	Color Index
A. B.	32	Hemorrhoids.....	2,144,000	20	0.47
J. S.	18	Bismuth poisoning.....	1,500,000	30	1
P. G.	30	Hemangioma of spleen.....	3,144,000	40	0.6
A. C.	32	Banti's disease.....	5,100,000	85	0.85
J. S.	28	Lead poisoning.....	4,640,000	70	0.7
L. Z.	34	Incomplete abortion.....	3,064,000	45	0.7
J. L.	25	Acute nephritis.....	2,888,000	50	0.9
W. H.	68	Carcinoma of stomach.....	4,010,000	70	0.9
H. S.	50	Chronic plumbism.....	3,680,000	70	0.9
L. O.	28	Hemorrhoids.....	2,500,000	30	0.6

What diagnostic value may be attached to the findings of bile pigment and bile salts in the plasma remains to be seen, but it is quite apparent that jaundice can be estimated with more certainty from observations of the plasma than from observations of the skin and sclerae. In the anemias, especially, a faint degree of jaundice, such as occurs in pernicious anemia, is frequently a matter of uncertainty, for the pallor exaggerates the normal pigmentation of the skin and the so-called lemon yellow skin is often a much disputed symptom. The large amount of bilirubin in the plasma in some cases increased the difficulties met in obtaining estimations of hemoglobin by the Tallqvist or Sahli method, for the adventitious yellow color prevents an accurate reading and tends to make the results too high. The diagnostic value of the presence of bile salts in the blood also is not very clear, but we

are inclined to accept such a finding as direct evidence of pernicious anemia in those cases in which anemia is a minor feature, and neurological signs and symptoms are the main features of the disease.

In the diagnosis of pernicious anemia we have relied principally on finding a marked anemia with a high color index associated in early cases, with parasthesias of the extremities, and other evidence of degeneration of peripheral nerves, and with degeneration of the posterior columns of the spinal cord in more advanced cases. In explanation of so large a series of pernicious anemia patients in twelve months in a clinic, such as is maintained at Lakeside Hospital, we can explain that some of them came by selection from a large private consulting practice.

As a method of prognosis, the measure of the jaundice of the plasma may be of considerable help, for it was found in three cases

#### —AND URINE IN SECONDARY ANEMIAS

Jaundice	History of Jaundice	Blood			Dialysis		Urine		
		Jaundice of Plasma	Gmelin's Test	Urobilin	Bile Pigment	Bile Salts	Bile Pigment	Bile Salts	Urobilin
+	+	0	0	0	0	0	0	0	0
0	0	5	0	0	0	0	0	0	0
0	+	15	0	0	0	0	0	0	0
+	+	40	+	0	0	+	0	0	+
0	0	0	0	0	0	+	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	10	0	0	0	0	0	0	0
0	0	10	0	0	0	0	0	0	0
0	0	15	0	0	0	+	0	0	0
0	0	0	0	0	0	0	0	0	0

that were observed, after a few months' interval, that the jaundice had diminished with the general improvement.

It is also seen that the patients kept on the ward and treated show a decrease in blood jaundice as they get relief from symptoms, the decrease usually being proportional to the increase in red cells.

#### SUMMARY

Bile pigment is frequently found in the blood in pernicious anemia, and is the cause of the jaundice. The bile pigment is in some way fixed to the plasma and therefore does not appear in the urine. The presence of bile pigment in the blood is not always betrayed by jaundice. Bile salts are frequently found in the blood, alone or associated with bile pigment. The jaundice in pernicious anemia is hepatic in origin.



# REFRACTOMETRIC STUDIES OF SERUM PROTEINS IN NEPHRITIS, CARDIAC DECOMPENSATION, DIABETES, ANEMIA, AND OTHER CHRONIC DISEASES\*

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In previous articles<sup>1</sup> we have reviewed the literature on serum proteins in health and disease, and have presented new values for serum proteins, obtained by Robertson's microrefractometric method, from normal adults and from those suffering with syphilis, pneumonia and other infections. The relation of globulin to the Wassermann reaction as well as the increase of serum proteins due to stasis have also been studied.

Former estimations of serum proteins in chronic diseases, which are referred to in a previous article,<sup>2</sup> show in a general way an increase of serum globulin with a decrease of total protein, the latter being most pronounced in hydremia. These results, though, are not uniform, owing to the use of varied technic and methods. We considered it necessary, therefore, to estimate the serum proteins in chronic diseases by the recent microrefractometric method of Robertson<sup>3</sup> which we have shown to be dependable and by which normal values have been already established.

## NEPHRITIS

The refractometer has been used for many years to estimate total serum proteins in nephritis, but it has never been used to quantitate the serum albumin and globulin. Strauss<sup>4</sup> used the refractometer to study nephritic serums and thought his results offered a guide to treatment, since they showed the amount of edema present. He claimed,<sup>5</sup> moreover, that cardiac edema never produced such low dilution of serum as nephritic edema, with which opinion Widal,

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1. Rowe, A. H.: *THE ARCHIVES INT. MED.*, 1916, **18**, 455; *Jour. Lab. and Clin. Med.*, 1916, **1**, 439. Tranter and Rowe: *Jour. Am. Med. Assn.*, 1915, **65**, 1433.

2. Rowe, A. H.: Footnote 1, first reference.

3. Robertson, T. B.: *Jour. Biol. Chem.*, 1915, **22**, 233.

4. Strauss: *Die Chronischen Nierentzündungen und ihre Einwirkung auf die Blut flüssigkeit*, 1902; *Therap. der Gegenw.*, 1903, **44**, 433; *Deutsch. med. Wchnschr.*, 1905, **31**, 83.

5. Strauss: *Ztschr. f. klin. Med.*, 1906, **60**, 501.

Bernard and Vaucher<sup>6</sup> agreed, while Reiss,<sup>7</sup> in a recent article, disagreed. We, on our part, have not found as low total proteins in cardiac edema as in nephritic edema. Brandenstein<sup>8</sup> and Georgopulos,<sup>9</sup> pupils of Strauss, continued the study of serums of edematous patients, showing, among other things, normal values in compensated cardiac disease and in chronic nephritis. Reiss also found little variation from normal in total proteins in chronic nephritis. He obtained marked dilution of blood serum in acute nephritis associated with edema and showed that this dilution occurred before and lasted after the subcutaneous edema was visible. The effect of salt on body weight and serum concentration was studied. Chiray<sup>10</sup> also studied by the use of the refractometer the serum dilution which occurs in cardiac and nephritic cases.

In grouping the cases of nephritis in the accompanying tables, the effect of edema and of uremia has been shown. No characteristic values for serum proteins which might aid in the clinical diagnosis of the type of kidney lesion were found.

Two cases of acute nephritis were included in Table 4 of a previous article.<sup>2</sup> In Case 5 of this table circumscribed edema was present, but not enough general edema to depress the total proteins below normal. In Case 6, on the contrary, generalized edema was present and the total proteins were markedly lowered. Both cases showed definite increase in the percentage of globulin and in the nonproteins.

The effect of chronic nephritis and edema on the serum proteins is shown in Table 1 of this article. The most marked variation from normal is the low total protein. This is due, in large part, to the presence of hydremia, though the toxic effect of retained waste products undoubtedly plays a rôle in reducing the available body proteins. Moreover the percentage of globulin and the nonproteins are above normal. Charts 1 and 2 show the striking way in which the total proteins and albumin rise and the nonproteins fall with the decrease of body weight due to the loss of edema. The separation of the albumin and globulin curves with recovery from the severe stage of illness is very pronounced and is well shown in both charts, especially in Chart 2, in which at the first examination the albumin and globulin were equal in amount and in ten days the ratio between the two became normal, that is, 26 per cent.

6. Widal, Bernard and Vaucher: *Semaine méd.*, 1911, **31**, 49.

7. Reiss: *Ergebn. d. inn. Med. u. Kinderh.*, 1913, **10**, 603; *Zentralbl. f. Kinderh.*, 1909, **14**, 150; *Jahrb. f. Kinderh.*, 1909, **70**, 311; *München. med. Wchnschr.*, 1908, **55**, 1853.

8. Brandenstein: *Zur Frage der Schädigung der Nierenkrankheiten durch Kochsalz*, Senator Festschrift, 1904.

9. Georgopulos: *Ztschr. f. klin. Med.*, 1906, **60**, 411.

10. Chiray: *Presse méd.*, 1908, **16**, 29.

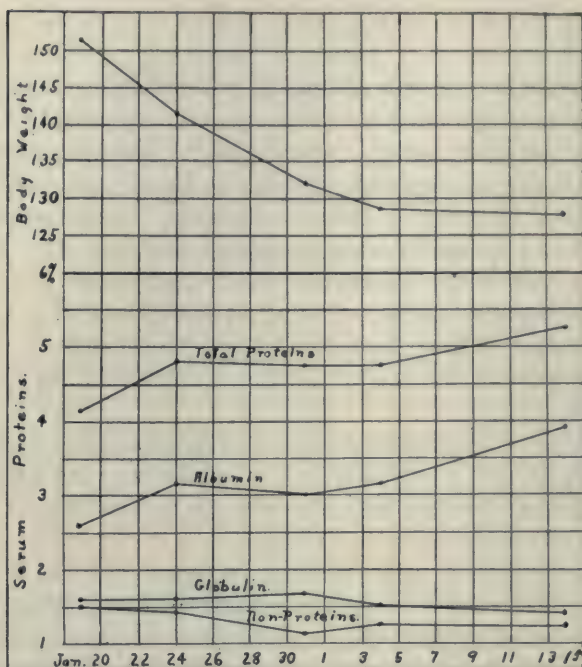


Chart 1.—Graphic results of Case 3 (Table 1). The low levels of serum albumin and total serum proteins in severe nephritis with edema followed by a rise in these values with clinical improvement and loss of edema is shown. The separation of the albumin and globulin curves in this chart is a definite index of recovery, though the failure of the total proteins to reach a more normal value indicates a bad prognosis.

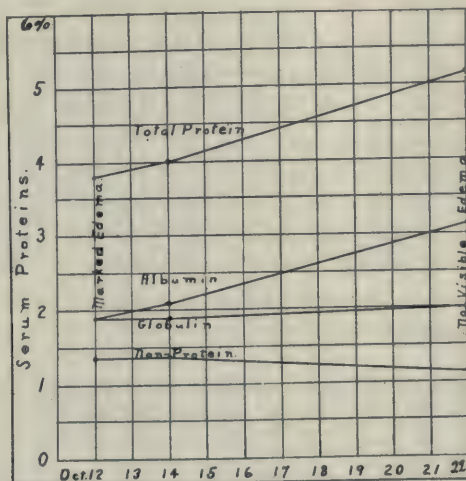


Chart 2.—Graphic results of Case 1 (Table 1). The comment of Chart 1 applies in this case. The separation of the albumin and globulin curves is most striking.



It is seen that the total protein at the last examinations made in Cases 1 and 3 were nearly 2 per cent. below the normal values, in spite of the complete absence of visible edema and a great loss of weight. This failure to return to the normal level is undoubtedly a manifestation of internal edema, which persists long after the subcutaneous edema is demonstrable, and also a manifestation of the chronic intoxication present in nephritis. That retained toxins can aid in breaking down serum proteins is certain. In this connection it is interesting to note that Castaigne and W. Chiray<sup>11</sup> have shown by

TABLE 1.—CHRONIC NEPHRITIS WITH EDEMA

Case No.	Age, Years	Date	Diagnosis, Data, etc.	Albumin	Globulin	Total Protein	Non-protein	Per Cent. of Globulin
1	34	10/12	Chronic nephritis with marked edema; slight rise of temperature and leukocytosis at entrance; Wassermann negative	1.9	1.9	3.8	1.4	50
		10/14	Nonprotein N = 56 mg. per 100 c.c. of blood; less edema	2.1	1.9	4	1.4	47.5
		10/22	No visible edema; walking around, feeling well	3.2	2	5.2	1.2	88
2	39	1/29	Chronic glomerular nephritis with marked edema; nonprotein N = 36 mg.; Wassermann wk. +; weight = 173 lbs.	3	1.6	4.6	1.2	85
		2/ 8	Less edema; no other improvement; weight = 162 lbs.	3	1.8	4.8	1.4	37.5
3	38	1/19	Chronic glomerular nephritis; marked edema; weight = 152 lbs.	2.6	1.6	4.2	1.5	88
		1/24	Edema disappearing with energetic eliminative treatment; weight = 144 lbs.	3.2	1.6	4.8	1.4	83
		1/31	Less edema; weight = 134 lbs.	3	1.7	4.7	1.2	36
		2/ 4	Edema practically gone; weight = 128 lbs.	3.2	1.5	4.7	1.8	82
		2/14	Edema gone; weight = 126 lbs.	3.9	1.4	5.3	1.8	26

the refractometer that subcutaneous injections of complex proteins cause slight diminutions in the serum proteins, which, they conclude, is due to the toxic effect of these injected substances. These authors, moreover, were able to produce cachexia and finally death by many repetitions of such toxic effects. Thus, in a case of nephritis whose serum proteins remain far below normal long after visible edema has disappeared we must assume a chronic toxicity. Reiss<sup>7</sup> learned to associate a bad prognosis with such cases. From the above considerations it is seen that the duration and extent of treatment of nephritics

11. Castaigne and Chiray, W.: *Compt. rend. Soc. de biol.*, 1906, **60-61**, 220.

could be guided in a definite way by the rapidity with which the total proteins return to their normal limits and by the separation of the albumin and globulin curves from one another. If either of these phenomena fail, prognosis is bad and treatment should be persistently continued.

Table 2 shows the effect of chronic nephritis with uremia on the serum proteins. Strauss<sup>4</sup> and Reiss<sup>7</sup> thought that the refractometric investigation of serum proteins was useless because of the great increase in the nonproteins. Reiss was certain that the correction of 0.6 per cent. which Widal, Bernard and Vaucher allowed for the increase in waste products in uremic serums was too small. Because of the fact that constant values have been used for nonproteins in determining the serum concentration with the refractometer, former

TABLE 2.—CHRONIC NEPHRITIS WITH UREMIA

Case No.	Age, Years	Diagnosis, Data, etc.	Albumin	Globulin	Total Protein	Non-protein	Per Cent. of Globulin
1	29	Chronic glomerular nephritis; hypertrophy and dilatation of heart, slight edema	3.4	2.4	5.8	1.7	41
2	45	Chronic glomerular nephritis, 11/15/16..	4.8	2.3	7.1	1.4	32
		In uremic coma for 18 hrs., 9/23/16....	■	1.9	7.9	1.55	24
3	22	Chronic glomerular nephritis; 160 mg. nonprotein N per 100 c.c. blood	3.9	2.2	6.1	2	36
4	45	Subacute nephritis; 86 mg. nonprotein N per 100 c.c. of blood; phenolsulphonaphthalein renal function = 15 per cent.	3.3	2.6	5.9	1.6	44
5	26	Chronic glomerular nephritis, beginning uremia; death 4 days later; Wassermann sl. +	5.5	2.1	7.6	1.75	28

results are not satisfactory. Thus Robertson's method, employing, as it does, a separate determination for nonproteins in each case, makes possible for the first time accurate determinations by the refractometer of serum proteins in cases of uremia.

Our results show that in uremic patients, the percentage of globulin is usually increased above normal. In those two patients nearest death the percentage of globulin was normal, which finding we are unable to explain. In this series the total proteins are normal in one half of the cases and moderately subnormal in the other half. These normal values indicate a dehydration of the body tissues due, in large part, to the inability to take any fluid into the system. The nonproteins in these cases are very high, as would be expected.

Table 3 shows the effect of chronic nephritis alone on the serum proteins. The majority of these results present a decided increase in

the percentage of globulin over the normal, though three of the values are within normal limits. The total proteins vary from normal to slightly subnormal values, due probably to the varying degrees of toxicity and internal edema present. The nonproteins vary from normal values to high abnormal values, which shows the varying degree of kidney damage present in different cases of chronic nephritis, and emphasizes the importance of laboratory investigation of all nephritics. This importance of laboratory results is best shown in Case 6, in which the total proteins are slightly below normal, the nonproteins and percentage of globulin markedly above normal, all of which variations point toward a likely return of the former uremic condition and suggest the importance of treatment in spite of the apparent health of the patient.

TABLE 3.—CHRONIC NEPHRITIS WITHOUT UREMIA OR EDEMA

Case No.	Age, Years	Diagnosis, Data, etc.	Albumin	Globulin	Total Protein	Non-protein	Per Cent. of Globulin
1	45	Chronic nephritis; chronic myocarditis; arteriosclerosis	4.4	2.5	6.9	1.2	86
2	19	Chronic nephritis; duodenal ulcer.....	6.1	1.8	7.9	1.1	23
3	26	Chronic glomerular nephritis.....	3.6	2.5	6.1	1.2	40
4	17	Chronic nephritis .....	3.8	2.3	6.1	1.4	38
5	40	Chronic glomerular nephritis; hypertrophy and dilation of heart; blood N = 40 mg.; phenolsulphonephthalein renal function = 25%; fundi showed marked hemorrhages and exudates	5.2	2.5	7.7	1.4	52
6	22	Chronic nephritis; 4 years before had uremic coma and albuminuric retinitis	3.9	2.4	6.3	1.8	38
7	34	Chronic nephritis; hypertrophy and dilation of heart; secondary anemia	4.8	1.3	6.1	1.6	21

#### CARDIAC DECOMPENSATION

The refractometric determinations of total proteins as done by Böhme and Reiss,<sup>7</sup> Widal,<sup>8</sup> Chiray<sup>10</sup> and others in cases of cardiac decompensation have shown certain interesting facts. The "internal edema," which may be present in spite of the absence of visible external edema, occurs in this condition as well as in nephritis. The fact that serum dilution is usually less in edematous cardiac patients than in edematous nephritic patients was commented on under the subject of nephritis. We have found, as did Böhme and Reiss, cases of generalized cardiac edema in which the total proteins are normal in amount. Chiray<sup>10</sup> also noticed this and offered as an explanation venous stasis, caused primarily by heart insufficiency, but helped by



TABLE 4.—CARDIAC DECOMPENSATION

Case No.	Age, Years	Diagnosis with Data	Albu- min	Glob- ulin	Total Pro- tein	Non- pro- tein	Per Cent. of Globulin
1	63	Decompensation of heart; arterio- sclerosis (general and renal); chronic passive congestion; edema in legs	4	1.9	5.9	1.4	34
2	43	Slight decompensation; mitral disease; auricular fibrillation; exophthalmic goiter? slight edema	4.7	2.6	7.3	1.3	36
3	38	Decompensation; auricular fibrillation; anasarca	4	2	6	1.3	33
4	20	Mitral stenosis; aortic and tricuspid insufficiency; pericardial effusion; 1/18/16, wt. = 130 lbs.	3.4	3.2	6.6	1.45	49
4	20	2/12/16, compensation regained; wt. = 128 lbs.	5	1.9	6.9	1.2	28
5	62	Myocardial insufficiency; arteriosclero- sis; pulsus alternans; edema	4.5	2.1	6.6	1.3	32
6	34	Myocardial insufficiency; tricuspid in- sufficiency; chronic mitral and aortic disease; no edema	4.6	2.7	7.3	1.5	37
7	67	Myocardial insufficiency, with edema; auricular fibrillation; chronic ne- phritis	4.3	2.1	6.4	1.2	33
8	32	Aortic and mitral insufficiency, with stenosis; edema	4	2	6	1.3	33
9	45	Decompensated hypertrophied and di- lated heart; edema; general arterio- sclerosis	3.8	2.5	6.3	1.3	40

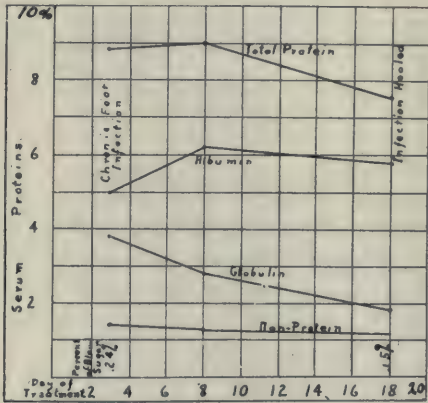


Chart 3.—Graphic results of Case 3 (Table 4). The high percentage of globulin due to a chronic foot infection in a diabetic with a fall of the globulin and separation of the albumin and globulin curves as the infection healed under the Allen starvation treatment is shown. The normal values for serum proteins obtained at the last examination are similar to those found in uncomplicated cases of diabetes.

kidney damage. This explanation has already been referred to in our recent article on venous stasis.<sup>12</sup> The percentage of globulin and the nonproteins are above normal in these cases of cardiac decompensation.

# ARTERIOSCLEROSIS

The patients studied in this series had arteriosclerosis as their primary diagnosis and all had hypertension. Total proteins were normal except for moderate decrease in Cases 3 and 4. The percentage of globulin, except in Cases 2, 3, and 5, were within normal limits. The nonproteins were normal except in Cases 1 and 3. When the average results are computed they are found to be only moderately abnormal.

TABLE 5.—ARTERIOSCLEROSIS

Case No.	Age, Years	Diagnosis	Albumin	Globulin	Total Protein	Non-protein	Per Cent. of Globulin
1	46	Arteriosclerosis, including coronary sclerosis	5	2.6	7.6	1.5	34
2	76	Arteriosclerosis; cardiosclerosis; partial heart block; retention cystitis	4.2	2.3	6.5	1.3	35
3	70	Arteriosclerosis; emaciation; gastric ulcer?	4.2	1.9	6.1	1.4	31
4	62	Arteriosclerosis .....	4.2	2.7	6	1.3	45
5	50	Arteriosclerosis .....	4.8	3.1	7.9	1.1	39
6	53	Arteriosclerosis; hemiplegia .....	5.5	1.8	7.3	1.3	25
7	50	Arteriosclerosis; chronic nephritis.....	5.3	1.9	7.2	1.3	26
8	53	Arteriosclerosis; chronic nephritis; hypertrophied and dilated heart	5.1	2.5	7.6	1.2	33
9	56	Arteriosclerosis .....	5	1.8	6.8	1.3	26

# DIABETES

Strubell,<sup>13</sup> Grober,<sup>14</sup> and Wagner<sup>15</sup> first used the refractometer to quantitate sugar in watery solutions and in diabetic urine. The latter application was not successful because of the many varying urinary constituents. Reiss<sup>16</sup> investigated serum proteins with the refractometer in later years, studying especially the relation of the serum concentration to the body weight and water balance in diabetes. He reviewed the literature on the blood concentration in diabetes. His conclusions

12. Rowe, A. H.: Footnote 1, second reference.

13. Strubell: *Verhandl. d. Cong. f. inn. Med.*, 1900, **18**, 417; *Deutsch. Arch. f. klin. med.*, 1901, **69**, 521; *München. med. Wchnschr.*, 1902, **49**, 616.

14. Grober: *Centralbl. f. inn. Med.*, 1900, **21**, 201.

15. Wagner: *Ueber quantitative Bestimmungen wässer zur Lösungen mit den Zeisschen Entauchrefractometer*, Dissertation, Jena, 1903.

16. Reiss: *Deutsch. Arch. f. klin. Med.*, 1909, **99**, 419; see also Footnote 7.

were that changes in weight in diabetes are due to anomalies in the water and salt excretion caused by an accompanying nephritis.

The results of our series of examinations in diabetics are shown in Table 6. With the exception of Case 3, the percentages of globulin are all normal. The results of Case 3 are shown in Chart 3. The first examination was made when there was an active chronic infection in the right foot. This accounts, undoubtedly, for the high percentage of globulin present, since as the condition improved with Allen treat-

TABLE 6.—DIABETES

Case No.	Sugar in Urine, %	Blood Sugar, %	Comment	Albumin	Globulin	Total Protein	Non-protein	Per Cent. of Globulin
1	4	0.22	Before Allen treatment was started	6	2	8	1.3	25
2	0.8	0.24	Tests made during period of lowered tolerance	6.2	1.9	8.1	1.3	23
3	0	0.24	Third day of starvation, 59.8 vol. per cent. CO <sub>2</sub> * bound by 100 c.c. of blood plasma; chronic infection in foot	5	3.8	8.8	1.4	48
	0	....	Eighth days of treatment, infection nearly well; low caloric intake	6.2	2.8	9	1.3	31
	0	0.15	Eighteenth day of Allen treatment, infection well; moderate caloric intake	5.8	1.8	7.6	1.2	24
4	0	....	Low caloric intake.....	5.7	1.3	7	1.3	19
5	0	....	Low caloric intake.....	5.5	1.7	7.2	1.2	24
6	0	....	Moderate caloric intake.....	6	1.9	7.9	1.2	24
7	2	0.36	Tuberculosis of the kidney with nephritis prevented a strict Allen treatment	5.6	1.8	7.4	1.4	24
8	0	....	Moderate caloric intake.....	5.7	1.5	7.2	1.3	21
9	0	....	Low caloric intake, 56.7 vol. per cent. of CO <sub>2</sub> bound by 100 c.c. of plasma	4.6	1.7	6.3	1.4	27
10	0	....	Starvation period; moderate degree of acidosis; 40.9 vol. per cent. of CO <sub>2</sub> bound by 100 c.c. blood plasma	4.2	1.5	5.7	1.2	26

\* The volume percentage of carbon dioxide was determined by Van Slyke's method.

ment and the foot finally healed, the globulin became normal. The improvement in this case is indicated, as in Charts 1 and 2, by a separation of the albumin and globulin curves. That the high percentage of globulin was not due to a high blood sugar content is shown by the fact that Cases 1, 2, and 7, which had as large amounts of blood sugar as did Case 3, had perfectly normal amounts of globulin. All examinations, except in Case 1, were made on patients receiving the Allen starvation treatment under the direction of Dr. Orville



Rogers. That the Allen treatment was not responsible for the normal amounts of globulin obtained in all cases except Case 3 is shown by the normal results obtained in Case 1.

The average value for nonproteins in this series is slightly higher than normal. It is interesting to notice that as improvement occurred in Case 3, the nonproteins decreased to normal limits. Cases 9 and 10 had low total proteins, which probably was a sign that hydremia was present as well as a diminution of the available body proteins due to the chronicity of the disease and the long duration of low caloric intakes. That hydremia was in part a cause for these low values is likely when we consider the frequency of edema during the starvation treatment, especially when salty broths are freely given.

TABLE 7.—ANEMIAS

Case No.	Diagnosis	Blood Picture			Albu- min	Glob- ulin	Total Pro- tein	Non- pro- tein	Per Cent. of Glob- ulin
		Red Cells	White Cells	Hemo- globin, %					
1	Pernicious anemia...	1,336,000	3,000	45	4.5	1.3	5.8	1.2	22
2	Pernicious anemia...	1,200,000	3,700	30	4.4	1.4	5.8	1.3	24
3	Pernicious anemia...	1,152,000	8,800	30	5	1.6	6.6	1.3	24
4	Secondary anemia— etiology?	3,896,000	7,500	35	4.8	1.5	6.3	1.3	23
5	Secondary anemia; probable malignancy	2,888,000	38,000	60	4.4	1.7	6.1	1.3	28
6	Banti's disease.....	4,800,000	10,100	75	5	1.6	6.6	1.25	24
7	Malignant endocarditis	4,240,000	10,200	75	2.9	2.7	5.6	1.35	48
8	Parenchymatous nephritis; marked edema	?	6,800	95	2.6	1.6	4.2	1.5	38
9	Chronic nephritis; marked edema	4,600,000	8,800	80	1.9	1.9	3.8	1.4	50

#### ANEMIAS

Reiss<sup>7</sup> reports the refractometric determinations of total proteins of serums in two cases of pernicious anemia, these results varying between 6.4 and 5.1 per cent., with typical low blood pictures. Martius<sup>17</sup> obtained similar results and commented on the fact that the serums were not as dilute as he expected they would be from the examination of the whole blood. The literature on pernicious anemia, reviewed in the preceding paper, showed in a general way this same condition, as well as no increase in the percentage of globulin. The three cases of our series yielded results which agree with those in the

17. Martius: *Folia haemat.*, 1906, **3**, 138.

literature. The serum proteins in secondary anemias due to cancer, Banti's disease, malignant endocarditis, and nephritis with edema are tabulated and are especially interesting in showing the remarkably high amount of total protein present in pernicious anemia as compared with the results in secondary anemias. A priori, one would assume that the lowest values for serum proteins would occur in pernicious anemia, in which one obtains the lowest number of red cells and very low percentages of hemoglobin. On the contrary, the lowest results occur where the red count and hemoglobin are much higher than in pernicious anemia.<sup>19</sup> In the last three cases of this series the percentage of globulin is high, as would be expected from the etiologic factors at work in the production of these anemias.

TABLE 8.—MISCELLANEOUS CHRONIC DISEASES

Case No.	Diagnosis, etc.	Albumin	Globulin	Total Protein	Nonprotein	Per Cent. of Globulin
1	Hyperthyroidism.....	5.4	2	7.4	1.2	27
2	Hyperthyroidism.....	5.7	1.1	6.8	1.2	16
3	Goiter (colloid).....	4.6	1.8	6.4	1.3	28
4	Hemophilia; R. C., 5,055,000; hemoglobin, 80%; bleeding time 2-2½ min.; clotting time 65 min.	5.9	2.5	8.4	1.3	30
5	Myeloma of spinal cord; Bence-Jones bodies in urine	4.8	2	6.8	1.5	29
6	Chronic bronchitis.....	5.7	2	7.7	1.3	26
7	Bronchial asthma.....	5.7	2	7.7	1.4	26
8	Pellagra.....	5.6	1.9	7.5	1.3	25
9	Obesity; Wassermann +++.....	6	1.6	7.6	1.2	21
10	Lead poisoning.....	5.5	1.8	7.3	1.1	25
11	Dyspepsia; constipation.....	5.4	2.1	7.5	1.2	28
12	Indigestion; gastric ptosis.....	5.3	1.7	7	1.1	24
13	Constipation.....	5.2	2	7.2	1	28
14	Duodenal ulcer.....	5	2.3	7.3	1.2	32
15	Cardiac neurosis.....	5.7	2	7.7	1.3	26
16	Neurasthenia.....	5	2.4	7.4	1.2	32

## MISCELLANEOUS CHRONIC DISEASES

The cases of hyperthyroidism, goiter (colloid), hemophilia, chronic bronchitis, pellagra, obesity, lead poisoning, chronic gastro-intestinal disorders, and neurasthenia presented normal values for serum proteins. One case of bronchial asthma and a case of myeloma of the spinal cord which showed the Bence-Jones bodies in the urine had

18. It is interesting to know that Reiss (Arch. f. exper. Path. u. Pharmakol., 1914, **51**, 19) found normal values for total proteins in severe cases of leukemia.

moderate elevation of the nonproteins, with normal values for the serum proteins. How much this elevation of nonproteins means in the case of myeloma we are unable to say. The single case of obesity examined showed triple positive Wassermann, but this syphilitic condition failed to elevate the globulin. Our normal results in a case of hemophilia coincide with those recently obtained by Hurwitz and Lucas<sup>19</sup> by Robertson's microrefractometric method. It is interesting to include a note about the finding by refractometric examination of profound changes in serum proteins by Widal<sup>20</sup> during the attacks of paroxysmal hemoglobinuria. It was impossible for us to confirm this and to study any possible changes in serum albumin and globulin that may occur in this disease.

#### SUMMARY

1. Chronic nephritis with edema shows the lowest values for total serum proteins obtained in disease. The cause of these low values is probably chronic intoxication as well as hydremia. The percentage of globulin and the nonproteins are definitely increased above normal limits.

2. Serum proteins in chronic nephritis with uremia have been determined for the first time by an accurate refractometric method. The total proteins are either normal or slightly above normal, due probably to dehydration. The percentage of globulin is usually increased, but approaches normal as death draws near. Nonproteins are extremely high.

3. Chronic nephritis without edema or uremia produces a decided increase in the percentage of globulin, a moderate increase in the nonproteins and slight depression of the total proteins.

4. Cardiac decompensation with or without edema shows a decrease of total proteins, though not to the extent found in chronic nephritis with edema. The percentages of globulin and the nonproteins are moderately above normal.

5. In arteriosclerosis the average results show normal total proteins, while nonproteins and percentages of globulin are moderately increased.

6. Diabetes shows normal percentages of globulin except when a complicating infection is present, which causes a rise. A slight increase in the nonproteins was found. Total proteins were normal except for subnormal values in two severe cases.

7. In pernicious anemia the total proteins are not as low as would be expected from examination of the whole blood, being higher than

19. Hurwitz and Lucas: *THE ARCHIVES INT. MED.*, 1916, **17**, 543.

20. Widal, Abrami, Brissaud, Bernard and Joltrain: *Arch. d. mal du Coeur*, 1915, **8**, 430.



in nephritis with edema, in which the blood picture is much nearer normal than in the former disease. Nonproteins and percentages of globulin are normal in pernicious anemia and in secondary anemias due to cancer and Banti's disease, but increased in the cases of secondary anemia due to infection and nephritis.

8. Hyperthyroidism, goiter (colloid), hemophilia, chronic bronchitis, pellagra, obesity, lead poisoning, chronic gastro-intestinal disorders and neurasthenia gave normal results. One case of bronchial asthma and one case of myeloma with Bence-Jones bodies showed moderate elevation of the nonproteins with normal values for the serum proteins.

9. The accompanying charts show how recovery from the given illness produces a rise in total proteins, a separation of the albumin and globulin curves or a fall of the nonproteins. It is suggested that these phenomena might be used to help determine prognosis and the duration of treatment.

## BACTERIOLOGIC STUDIES IN SUBACUTE STREPTOCOCCUS ENDOCARDITIS \*

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It was the purpose of this study to search for distinctive features in the group of streptococci associated with subacute streptococcus endocarditis. The criteria adopted were cultural, biochemical and immunologic characteristics. Incidentally, the presence of antibodies in the blood of patients suffering from the disease and the immediate effect of blood transfusions on the bacteriemia were investigated.

The recognition of a distinct form of endocarditis, called by some writers subacute bacterial, by others endocarditis lenta and chronic infectious endocarditis, has been of comparatively recent date and of slow evolution. Although of only moderately common occurrence, the disease is extremely important, since all of the cases studied, except one,<sup>1</sup> have been fatal.

Virchow in 1855 first suggested an infectious origin for the endocarditis associated with uterine infections, and attention was thereafter directed to the finding of bacteria in the vegetations in both ulcerative and verrucous endocarditis. Heiberg<sup>2</sup> described a case of acute endocarditis which had been reported by Winge in 1869 in which micrococci were found in the vegetations and added another case of acute ulcerative endocarditis in which small micrococci were present in the vegetations.

For a short period it was thought that bacteria were present in all forms of endocarditis. Thus, Klebs<sup>3</sup> felt that he could separate rheumatic from septic cases by the character of the cocci found in microscopic sections. Köster<sup>4</sup> thought that verrucous endocarditis was a chronic and healed stage of ulcerative endocarditis and hence originally of bacterial origin. It may be noted that this idea of healed bacteria-free endocarditis has been supported with new evidence in recent years by Libman,<sup>5</sup> but with the distinction that the healed cases are supposed to have been of the subacute bacterial type.

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\* From the Medical Clinic, Presbyterian Hospital, Columbia University, New York.

1. Hemsted, H.: *Lancet*, London, 1913, **1**, 10.

2. Heiberg, H.: *Virchows Arch. f. path. Anat.*, 1872, **56**, 407.

3. Klebs: *Arch. f. exper. Path. u. Pharmakol.*, 1878, **9**, 52, cited by Wyssokowitch (Footnote 8).

4. Köster, K.: *Virchows Arch. f. path. Anat.*, 1878, **72**, 257.

5. Libman, E.: *Am. Jour. Med. Sc.*, 1913, **146**, 525.

Later investigators failed to demonstrate bacteria in the lesions of verrucous endocarditis. Hamburger<sup>6</sup> examined ten such cases with negative results and Weichselbaum<sup>7</sup> questioned whether verrucous endocarditis was due to microbic invasion. This view received further support from Wyssokowitch<sup>8</sup> and Orth.<sup>9</sup>

Additional interest was lent to the discussion by the experimental production of acute endocarditis by previously puncturing the aortic leaflets according to a method devised by Rosenbach.<sup>10</sup> Wyssokowitch<sup>8</sup> was able to infect the injured valve by injecting pyogenic streptococci. Ribbert<sup>11</sup> produced acute endocarditis merely by injecting a suspension of staphylococci and fine particles of potato intravenously. Many reports of the production of endocarditis by injecting cultures of streptococci have been subsequently published.

As the facts then stood, there were two general types of endocarditis, namely, those associated with bacteria and those in which no direct connection with bacteria could be proved. The introduction of blood culture methods was responsible for separating the bacterial group into acute and chronic cases. Of the early reports, that of Kühnau<sup>12</sup> is noteworthy, in that sixty-six cases of rheumatism, many of which were accompanied by endocarditis, gave negative cultures, the finding of sarcinae, staphylococci and hay bacilli being discounted.

It soon became evident that among those cases of endocarditis which gave definitely positive blood cultures a certain group could be distinguished because of their subacute or chronic character. These cases most frequently yielded streptococci from blood cultures. This was noted by Harbitz.<sup>13</sup> Later Horder<sup>14</sup> very accurately described cases of acute and chronic infectious endocarditis of which seventeen were subacute and chronic and yielded blood cultures of "saprophytic streptococci." Osler at the same time pointed out the importance of the latter group. In the following year Schottmüller<sup>15</sup> individualized this type of endocarditis with the name "endocarditis lenta," a disease of slow, insidious onset, definite bacteriology and symptoms of injured heart valves with bacteriemia. The five cases which he described were caused by streptococci of the green-producing variety. In the

6. Hamburger: Ueber akute Endocarditis in ihrer Beziehung zu Bakterien, Berlin, 1879, cited by Wyssokowitch (Footnote 8).

7. Weichselbaum: Centralbl. f. Bakteriöl., 1887, **2**, 209.

8. Wyssokowitch, W.: Virchows Arch. f. path. Anat., 1886, **103**, 301.

9. Orth, J.: Virchows Arch. f. path. Anat., 1886, **103**, 333.

10. Rosenbach, O.: Arch. f. exper. Path. u. Pharmakol., 1878, **9**, 1, cited by Wyssokowitch (Footnote 8).

11. Ribbert: Deutsch. med. Wchnschr., 1885, **11**, 717.

12. Kühnau, W.: Ztschr. f. Hyg. u. Infektionskrankh., 1897, **25**, 492.

13. Harbitz, F.: Deutsch. med. Wchnschr., 1899, **25**, 121.

14. Horder, T. J.: Quart. Jour. Med., 1908-1909, **2**, 289.

15. Schottmüller, H.: München. med. Wchnschr., 1910, **57**, 617.



same year Libman and Celler<sup>16</sup> described the same disease under the name of "subacute bacterial" endocarditis and found later<sup>17</sup> that while most of the streptococci produced green on blood agar plates, some were indifferent and some produced slight clearing. They also stated that the streptococci found were not very virulent.

The fact that positive blood cultures are occasionally obtained in rheumatic endocarditis should not confuse the conception of the disease under discussion, because bacteriemia in rheumatic endocarditis is rare, slight and transitory when it does occur, or may be present just before death. Subacute bacterial endocarditis, on the other hand, is a disease in which bacteriemia is a constant feature. This question is well discussed in a review by Simons.<sup>18</sup>

While the bacteria obtained are almost always streptococci, other organisms, such as influenza bacilli and gram-negative cocci have been described. The cases on which the present study was based were all cases of streptococcus infection. The characteristics of these streptococci have been studied by others, and such features as low virulence and their effect on blood agar have been described. Horder<sup>14</sup> studied the fermentation reactions of the streptococci from seventeen such cases from the standpoint of the grouping proposed by Andrewes and Horder<sup>19</sup> and found that nine belonged to the salivarius type and eight to the fecalis type. Major<sup>20</sup> studied the complement fixing antibodies in the serum of a patient and concluded that the reaction was not specific, since it was also obtained with an antigen made from *Streptococcus pyogenes*. Rosenow<sup>21</sup> claims that the bacteria usually isolated from these cases are "modified pneumococci," and tries to show that "the continuation of infection and death are largely due to a process of bacterial immunization against the antibodies of the host rather than to the virulence in the usual sense of the infecting bacteria."

#### CASE REPORTS

The twelve cases herein described were typical of the disease. A brief synopsis of each case follows:

CASE 1.—R. T., ironworker, aged 43, gave an indefinite history of rheumatism. He had "typhoid-pneumonia" twenty years previously. He had pains about the heart for twenty years. The onset of the present trouble was gradual, with weakness and chills. Later features were petechiae, subcutaneous nodules, arthritis in the small joints of hands and feet, left-side cardiac enlargement and signs of aortic and mitral insufficiency. The Wassermann reaction was negative.

16. Libman, E., and Celler, H. L.: Am. Jour. Med. Sc., 1910, **140**, 516.

17. Libman, E.: Am. Jour. Med. Sc., 1912, **144**, 313.

18. Simons, I.: Quart. Jour. Med., 1913-1914, **7**, 291.

19. Andrewes, F. W., and Horder, T. J.: Lancet, London, 1906, **2**, 775.

20. Major, R. H.: Bull. Johns Hopkins Hosp., 1912, **23**, 326.

21. Rosenow, E. C.: Jour. Infect. Dis., 1909, **6**, 245; *ibid.*, 1910, **7**, 429.

The leukocytes varied from 10,000 to 15,000, the temperature from 99 to 100. *Streptococcus A* 4 was isolated repeatedly from blood cultures during life and from the blood and spleen postmortem. Six months before death there were sixteen colonies per cubic centimeter and a few days before death 165. The duration was thirteen months.

CASE 2.—T. O., a carpenter, aged 34, gave a history of chorea, "malaria" and gonorrhea, but not of rheumatism. The onset of the present illness was gradual, with weakness, fever, chills and signs of aortic and mitral regurgitation. He was under observation only twelve days before death. The same streptococcus *O* was recovered in pure culture three times. The total duration was about two months.

CASE 3.—C. S., a laundryman, aged 42, had sinus infection several months before admission. The onset of his present illness was gradual, with weakness. The features were purpuric eruption, necrotic area on the feet and lower legs and terminal hemiplegia. There were signs of marked aortic regurgitation. The leukocytes numbered from 25,000 to 35,000. The Wassermann reaction was negative. The temperature ranged from 99 to 103. *Streptococcus A* 30 was recovered repeatedly. There were forty-three colonies per cubic centimeter. The duration of the illness was about four months.

CASE 4.—M. S., a married woman, aged 31, gave a history of "inflammatory rheumatism" at the age of 15 and again at 25. The onset of this illness was slow, with "muscular rheumatism." After seven weeks she began to have fever, joint pains and palpitation. She was in bed six weeks longer. She entered the hospital with signs of marked mitral insufficiency, tenderness in the small joints of the hand, leukocytosis of from 12,000 to 16,000, and temperature of from 101 to 104. *Streptococcus XK* was isolated three times during three weeks, at the end of which time the patient died of cerebral embolism. The total duration of the illness was about four months.

CASE 5.—M. H., a single woman, aged 36, gave a history of scarlatina, diphtheria and frequent sore throat in childhood. She had acute articular rheumatism at 21, accompanied by "heart trouble." There had been dyspnea for the previous four years. The onset of this illness was gradual, with joint pains, chills, weakness and palpitation. The clinical features were petechiae, enlarged spleen and signs of aortic obstruction and regurgitation and mitral regurgitation. The Wassermann reaction was negative. The leukocytes varied from 6,000 to 15,000, and the temperature from 101 to 104. *Streptococcus B* 29 was isolated repeatedly during life, 175 colonies per cubic centimeter, and in pure culture from the aortic vegetations postmortem.

CASE 6.—M. R., a junk dealer, aged 46, gave no history of previous infections of any kind. Rheumatism was denied. The onset of his illness was very insidious, with weakness, fever and occasional sweat. Examination revealed signs of aortic insufficiency. Later there were petechiae. The leukocytes varied from 12,000 to 15,000, the temperature from 100 to 102. The Wassermann reaction was negative. *Streptococcus "R"* was isolated four times over a period of two months. The patient died of cerebral embolism. The total duration of the illness was about three months.

CASE 7.—F. M., a married woman, aged 22, had measles in childhood, chorea at 6, diphtheria at 12, and rheumatism at 12. She had had palpitation from the age of 7. She was said to have had "aortic regurgitation" for years. The onset of this illness occurred with headache, weakness and gastro-intestinal disturbance. The clinical features were pain in the left shoulder and signs of aortic and mitral insufficiency. The leukocytes numbered 7,000, and the temperature varied from 101 to 103. The Wassermann reaction was negative. *Streptococcus A* 148, 170 colonies per cubic centimeter, was recovered repeatedly during life and from the blood after death. The duration of the illness was about two and a half months.



CASE 8.—J. M., a boy, aged 18, gave a history of acute articular rheumatism at 12 and 16 years, accompanied by "heart trouble." The onset of his illness was gradual, with palpitation, precordial pain and joint pains. The clinical features were a tender joint of one of the small toes and signs of aortic and mitral insufficiency. The leukocytes numbered 18,000. The Wassermann reaction was negative. The temperature varied from 101 to 103. *Streptococcus B 4* was isolated repeatedly, 570 colonies per cubic centimeter. The patient died after an illness of about five months.

CASE 9.—R. M. B., a physician, aged 34, gave a history of acute articular rheumatism at the age of 13. He was known to have had "aortic regurgitation" for ten years. The onset of this illness was gradual, with weakness, continuous fever and joint pains. The clinical features were signs of aortic insufficiency, enlarged spleen, arthritis of the small joints of the hand, petechiae and subcutaneous nodules. *Streptococcus MB* was isolated four times in pure culture, from four to six colonies per cubic centimeter, over a period of four months. The duration of his illness was seven months. Pure culture of streptococci were obtained postmortem from the heart's blood and from the spleen; and streptococci were present in the smears of aortic vegetation.

CASE 10.—P. S., a boy, aged 15, had scarlet fever followed by "swelling of the legs" at 4; joint pains and "heart trouble" at 6; attack of cardiac weakness with fever at 8; similar attack with pain in the feet, neck and abdomen with sterile blood culture at 11. The present trouble began with pain in feet, ankle and knee, with petechiae, subcutaneous nodules and progressive weakness. The clinical features were tenderness over the spleen, petechiae and signs of aortic and mitral insufficiency. The leukocytes numbered 14,000. The temperature ranged from 99 to 102. The Wassermann reaction was negative. *Streptococcus A 26* was isolated frequently over a period of ten months, there being seventeen colonies per cubic centimeter. The duration of the illness was about eleven months.

CASE 11.—C. C., laundryman, aged 36, gave a history of occasional sore throat. There was a slow onset of the present illness, with continuous fatigue, occasional chill, loss of weight and cough. The clinical features were signs of marked aortic insufficiency. The temperature ranged from 98 to 102. The leukocytes numbered from 8,000 to 10,000. The Wassermann reaction was negative. *Streptococcus A 56* was isolated repeatedly, from 165 to 200 colonies per cubic centimeter. The duration of the illness was about eight months.

CASE 12.—A. B., a married woman, aged 33, gave a history of scarlatina and diphtheria in childhood and "malaria" with "pneumonia" at 15. There was a vague history of rheumatism. The onset of the present illness was with sore throat, followed by chills and later, by joint pains and painful finger tips. The clinical features were petechiae, subcutaneous nodules, pain in the hips and signs of mitral insufficiency and stenosis. The leukocytes numbered 15,000. The Wassermann reaction was negative. The temperature ranged from 99 to 103. *Streptococcus A 32* was isolated frequently during three months, from 500 to 1,200 colonies per cubic centimeter. The patient died about four months after the onset.

#### METHODS

1. Blood Cultures: From 30 to 40 c.c. of blood were withdrawn from a cubital vein by means of a McRae needle. The blood was planted directly in flasks of broth, from 5 to 10 c.c. of blood being used. Dextrose agar plates were poured (from 2 to 3 c.c. of blood each) and 5 c.c. were hemolyzed in 40 c.c. of sterile distilled water and the sediment, after centrifugalization, was planted in a deep tube of



melted ascitic dextrose agar and allowed to harden before incubating. For the purpose of noting the effect of transfusion on the number of colonies per cubic centimeter plates were poured with a definite amount of blood just before and at intervals after the transfusion.

2. For the purpose of demonstrating antibodies in the serums of patients or animals, agglutination reactions, "thread" reactions, and complement fixation reactions were done as follows:

a. Agglutination Reactions: Dilutions of serum were made in tubes containing 0.5 c.c. dextrose broth, so that the first tube contained 0.1 c.c. of serum, the second 0.05 c.c., the third 0.025 c.c., etc. To each tube 0.5 c.c. of dextrose broth culture was added. The mixtures were placed in the water bath at 37 C. for two hours and readings were made after the tubes had remained on ice over night.

b. "Thread" reactions were done by making dilutions of serum in tubes containing 1 c.c. dextrose broth so that the first tube contained 0.1 c.c. of serum; the second 0.01 c.c., the third 0.001 c.c., etc. The tubes were then inoculated with a drop of active dextrose broth culture and allowed to incubate for twelve hours. When the reaction was positive, the growth was restricted to an agglutinated pellicle in the bottom of the tube. Tubes containing normal serum were used as controls. The "thread reaction" was originally employed by Pfaundler<sup>22</sup> for testing the agglutinin content of antityphoid serums and has been further developed by Mandelbaum,<sup>23</sup> Gaehstgens and Kamm<sup>24</sup> and Dennemark.<sup>25</sup>

c. Complement Fixation Reactions: Two units of complement and antishcep hemolytic amboceptor were used with 0.5 c.c. of 5 per cent. saline suspension of sheep cells, 0.1 c.c. of patient's serum, 0.1 c.c. antigen and saline enough to make the final volume 2.5 c.c. The antigen was prepared as follows: The washed sediment of 125 c.c. of a twenty-four-hour broth culture was emulsified in 5 c.c. saline and precipitated by the addition of 5 c.c. absolute alcohol. After centrifugalization the supernatant fluid was discarded and the precipitate dried in vacuo, ground and weighed. Ten mg. were dissolved in 5 c.c. of 2 per cent. antiformin at 56 C. and neutralized with normal sulphuric acid, litmus paper being used as an indicator. The free chlorin was liberated by adding one or two drops of 5 per cent. sodium thio-sulphate solution, the end result being tested with potassium iodid starch paper. The solution was then made up to 10 c.c. with carbolyzed saline so that 1 c.c. contained 1 mg. of dried precipitated bacteria.

22. Pfaundler, M.: *Centralbl. f. Bakteriol.*, 1898, **23**, 131.

23. Mandelbaum, M.: *München. med. Wchnschr.*, 1910, **57**, 178.

24. Gaehstgens, W., and Kamm, W.: *München. med. Wchnschr.*, 1910, **57**, 1389.

25. Dennemark: *Centralbl. f. Bakteriol.*, 1911, **58**, 354.

3. The cultural characteristics of the streptococci included (a) their appearance in broth, (b) their effect on serum dextrose agar, (c) their solubility in bile, (d) the lethal dose of a twenty-four-hour broth culture for white mice, and (e) their effect on red blood cells. The last effect was tested by planting cultures on blood agar plates and by mixing a twenty-four-hour broth culture with a 5 per cent. saline suspension of sheep red blood corpuscles according to the method recommended by Lyall.<sup>26</sup> (f) The fermentation reactions on litmus milk and on raffinose, inulin, salicin and mannite serum water mediums were studied. Each strain was started from a single colony.

4. For the purpose of attempting a classification of the streptococci on an immunologic basis, rabbits were inoculated with saline emulsions of killed streptococci at four-day intervals in doses equivalent to 10 c.c. broth culture. The injections were continued until the serum of an animal showed marked complement-binding capacity for the corresponding streptococcus antigen. Then the serum was tested against the other strains for cross fixation and cross agglutination.

#### RESULTS

1. Blood cultures: (a) As indicated in the case reports, each patient repeatedly yielded a positive blood culture. The colonies were always present in all the mediums; the streptococcus isolated was always in pure culture and identical with the organisms of each of the other cultures in the same case. The number of colonies varied from five to 1,200 per cubic centimeter. The bacteriemia did not fluctuate or change with rapidity and the same count might be expected from day to day. However, there seemed to be a gradual increase in the number of colonies per cubic centimeter as the disease advanced. The degree of bacteriemia did not seem to bear any direct relationship to the duration of the disease.

b. Effect of transfusion on the bacteriemia (Table 1): In four patients the effect of transfusion on the number of streptococci in the circulating blood was studied. Two different methods of transfusion were employed: (1) The Lindemann syringe method, (2) defibrination of the blood by wire or beads immediately on withdrawal from the donor, filtration through gauze and injection without the addition of saline. In the Lindemann method normal salt solution was injected to determine whether the needle was properly placed and at intervals during the transfusion when difficulty was experienced in securing blood from the donor. The syringes were also washed frequently with saline. The saline used in these transfusions had stood in the flasks several days after sterilization and the water from which the saline

26. Lyall, H. W.: Jour. Med. Research, 1914, **30**, 487.

was prepared was not freshly distilled. It was later shown that the severe reactions following transfusions could be largely eliminated by the use of freshly prepared saline. In Cases 1 and 10 the transfusions with accompanying febrile reactions were followed by complete sterilization of the blood for twenty-four hours. In Case 11 transfusion with defibrinated blood on three different occasions was unattended by febrile or other unpleasant reactions and the bacteriemia was not affected. In Case 12 the two facts illustrated by the three previous investigations was confirmed, but the additional evidence was brought forward that the injection of 300 c.c. of impure saline was followed by a severe febrile reaction. This resulted in as marked temporary reduction in the degree of bacteriemia as followed the injection of blood

TABLE 1.—EFFECT OF TRANSFUSIONS ON BACTERIEMIA

Case No.	Streptococcus	Transfusion with	Febrile Reaction	Colonies per Cubic Centimeter				
				Before	At Time of Reaction	24 Hours	48 Hours	72 Hours
1	A 4	Whole blood, Lindemann method	Severe	8	....	0	....	9
		Whole blood, Lindemann method	Severe	25	....	0	....	34
10	A 26	Whole blood, Lindemann method	Moderate	17	....	0	....	8
11	A 56	Defibrinated blood....	None	135	....	135		
		Defibrinated blood....	None	195	....	123		
		Defibrinated blood....	None	165	165			
12	A 32	Whole blood.....	Severe	1,200	800	15		
		Defibrinated blood....	None	1,200	....	920	....	1,500
		300 c.c. saline.....	Severe	750	40			

and saline. It seems evident, therefore, that the reduction in bacterial count is due to the reaction on the part of the patient and not to the introduction of complementary substances or antibodies in the donor's blood.

2. Immune Bodies in Patient's Serum (Table 2): The serum of each patient was tested with one or two of the immune reactions as shown in Table 2. The organisms A 56 and A 32 grew in an agglutinated mass so that the agglutination or thread reaction could not be applied to the serum in Cases 11 and 12.

It will be seen that all the serums contained antibodies against the homologous organisms. This is most markedly exemplified in the thread reaction, in which the tendency to grow in clumps in dilutions of immune serum was most striking.



TABLE 2.—IMMUNE REACTIONS WITH PATIENTS' SERUM AGAINST HOMOLOGOUS STREPTOCOCCI

Case No.	Streptococcus	Agglutination Reaction	Thread Reaction	Complement Fixation Reaction
1	A 4	.....	1-10 +++ 1-100 ++ 1-1000 +	
2	O	1-10 + 1-20 + 1-40 +		
3	X K	.....	1-10 ++ 1-100 ++ 1-1000 +	
4	A 30	1-10 ++ 1-20 ++ 1-40 +	1-10 +++ 1-100 ++ 1-1000 +	
5	B 29	.....	1-10 ++ 1-100 + 1-1000 +	0.1 c.c. ++ 0.05 c.c. — —
6	R	1-10 + 1-20 + 1-40 ±	1-10 + 1-100 + 1-1000 +	
7	A 148	1-10 + 1-20 + 1-140 +	1-10 ++ 1-100 ++ 1-1000 +	0.1 c.c. —
8	B 4	.....	1-10 +++ 1-100 ++ 1-1000 +	0.1 c.c. + 0.05 c.c. + 0.025 c.c. —
9	M B	.....	1-10 ++ 1-100 ++ 1-1000 —	
10	A 26	.....	1-10 +++ 1-100 ++ 1-1000 +	0.1 c.c. ++++ 0.05 c.c. ++++ 0.025 c.c. ++++

3. The cultural characteristics and virulence for white mice are shown in Table 3. It will be seen that not all the organisms can be termed viridans, since four were indifferent in their action on blood cells. The term "mitis" is appropriate, since lack of virulence is a characteristic of all the strains. It is noteworthy that the four indifferent strains grew with clear supernatant broth and showed short chains and a disposition to clump. Among the strains which grew with turbidity the sediment was more granular when first isolated than later, due possibly to the presence of agglutinating substances carried from the blood of the patient by the bacterial bodies through two or three generations.

4. Classification: This was considered from the standpoints of fermentation, agglutination and complement fixation reactions.

a. The fermentation reactions are given in Table 4, in which the organisms are arranged according to groups. All of the strains, except A 32, which was lost, were tested when first isolated and again after an interval of from three to eighteen months. Only one organ-

TABLE 3.—CULTURAL CHARACTERISTICS

Case No.	Strepto-coccus	Growth in Plain Broth	Aseptic Dextrose Agar	Bile Solubility	Effect on Red Blood Cells	Lethal Dose for White Mice
1	A 4	Turbid; moderately long chain	Precipitated	Insoluble	Green	Not fatal at 2 c.c.
2	O	Turbid; moderately long chain	Precipitated	Insoluble	Green	Not fatal at 2 c.c.
3	X K	Turbid; moderately long chain	Precipitated	Insoluble	Green	Not fatal at 2 c.c.
4	A 30	Turbid; moderately long chain	Precipitated	Insoluble	Green	Not fatal at 2 c.c.
5	B 29	Turbid; moderately long chain	Precipitated	Insoluble	Green	Not fatal at 2 c.c.
6	R	Turbid; moderately long chain	Precipitated	Insoluble	Green	Not fatal at 2 c.c.
7	A 148	Turbid; moderately long chain	Precipitated	Insoluble	Green	Not fatal at 2 c.c.
8	B 4	Turbid; moderately long chain	Precipitated	Insoluble	Green	Not fatal at 2 c.c.
9	M B	Granular sediment; clumps	Precipitated	Insoluble	Indifferent	Not fatal at 2 c.c.
10	A 26	Soft white sediment; clumps	Precipitated	Insoluble	Indifferent	Not fatal at 2 c.c.
11	A 56	Flocculent sediment; clumps	Precipitated	Insoluble	Indifferent	Not fatal at 2 c.c.
12	A 32	Granular sediment; clumps	Precipitated	Insoluble	Indifferent	Not fatal at 2 c.c.

TABLE 4.—FERMENTATION REACTIONS \*

Strepto-coccus	Time in Months Between 1st and 2d Test	Number of Generations between 1st and 2d Test	Milk	Raffinose	Inulin	Salicin	Mannite
A 4	18	10	+	—	—	—	—
O	15	4	+	—	—	—	—
X K	11	5	+	—	—	—	—
A 30	18	10	+	+	—	—	—
B 29	8	8	+	+	—	—	—
R	18	7	+	+	—	—	—
A 148	14	5	+	+	—	—	—
B 4	8	2	+	—	+	+	—
M B	8	8	+	—	—	+	—
A 26	15	10	+	—	—	+	+
A 56	15	8	+	+	+	—	+
A 32	..	..	±	—	—	—	—

\* In this table + means acid and clot; ± means acid and no clot. The reactions given in the table are those given recently by strain A 148.

ism, *A* 148, changed; it fermented inulin and salicin when first isolated and twelve months later fermented only raffinose on three different trials. The later reading is indicated in the table. This change in fermentation reaction does not remove it from the *salivarius* group. According to this table, there are two members of the *fecalis* group, the remainder being members of the *salivarius* group.

*b.* Complement Fixations: A rabbit was immunized to each strain, except *A* 32, which was lost; an antigen, however, was made of some dried sediment of *A* 32 and tested against a few of the immune serums. Five out of the eleven rabbits' serums were tested before immunization and gave no fixation with any of the eleven antigens.

The relations of the streptococci are given in Table 5. It will be noted that *A* 26, *M B*, *A* 56 and *A* 32, which are indifferent in their action on blood cells, fall (as far as the limited tests on *A* 32 can be of value) more or less into a common group. Moreover, *A* 26 and *A* 56 are the mannite fermenters. The raffinose fermenters are fixed, variously, by serums of animals immunized by members of the other groups. There is no obvious relationship between the grouping made on the basis of fermentation reactions and that on the basis of complement fixation reactions. Viewed from the standpoint of complement fixation reactions alone, it will be seen that the strains arrange themselves in two general groups—*A* 26, *M B*, *A* 56, *A* 32; and *R*, *X K*, *A* 30, *B* 29 and *O*; with members *A* 148 and *B* 4 forming strong bonds between. Yet the members in the upper and lower portions of the chart can be said to be independent.

*c.* The serums of the same rabbits, of the same or nearly the same dates, were used in the agglutination reactions as were employed in the complement fixation reactions. It soon became apparent that agglutination was confined to a narrower zone than complement fixation and was more likely to be restricted to homologous serums and bacteria. It will be seen that the only discrepancy is in the case of streptococcus *R*, which is agglutinated by serum *B* 4, whereas it is fixed only by its homologous serum; and it will be further noticed that serum *R* agglutinates streptococcus *B* 4, but does not give fixation with *B* 4. Accordingly the boundary line established by the agglutination reaction is probably the more correct in this case. -

#### COMMENT

A résumé of the various studies in classification shows that the organisms all fall definitely into the nonhemolytic streptococcus group. According to their action on red blood cells they fall into two subgroups, namely, green producers, so-called viridans, and indifferent strains, so-called saprophiticus. The last-named strains all gave a



TABLE 5.—COMPLEMENT FIXATION REACTIONS \*

Rabbit Serums Im- mune to Streptococcus	A 4	A 26	M B	A 56	A 32	A 148	B 4	R	X K	A 30	B 29	O
A 4.....	+	—	—	—	0	—	—	—	—	—	—	—
A 26.....	+	+	+	—	—	—	—	—	—	—	—	—
M B.....	—	±	+	+	+	+	+	—	—	—	—	—
A 56.....	—	±	+	+	—	+	+	—	—	—	—	—
A 32.....	0	0	0	0	0	0	0	0	0	0	0	0
A 148.....	—	—	—	—	0	+	+	—	—	—	—	—
B 4.....	—	—	—	—	0	±	+	—	—	—	—	—
R.....	—	—	—	—	0	—	—	+	—	—	—	—
X K.....	—	—	—	—	0	+	+	+	+	—	—	—
A 30.....	—	—	—	—	0	±	±	—	—	±	±	—
B 29.....	—	—	—	—	0	—	—	+	—	+	+	±
O.....	—	—	—	—	0	—	—	+	—	+	+	+

\* In this table + indicates reactions of practically the same strength with homologous and heterologous antigens; ± indicates that reactions were only weakly positive; — indicates negative reactions; and 0 indicates reactions not done.

TABLE 6.—AGGLUTINATION REACTIONS \*

Rabbit Serums Im- mune to Streptococcus	A 4	A 26	M B	A 56	A 32	A 148	B 4	R	X K	A 30	B 29	O
A 4.....	+++	—	—	—	0	—	—	—	—	—	—	—
A 26.....	—	++ ++	—	—	0	—	—	—	—	—	—	—
M B.....	—	—	++ +	+	0	—	—	—	—	—	—	—
A 56.....	—	—	—	++ +	0	—	—	—	—	—	—	—
A 32.....	0	0	0	0	0	0	0	0	0	0	0	0
A 148.....	—	—	—	—	0	++	++	—	—	—	—	—
B 4.....	—	—	—	—	0	++	++ ++	++ +	—	—	—	—
R.....	—	—	—	—	0	—	++ +	++ +	—	—	—	—
X K.....	—	—	—	—	0	—	++	—	++	—	—	—
A 30.....	—	—	—	—	0	+++	++ +	++	—	+++	—	—
B 29.....	—	—	—	—	0	—	—	—	—	—	+++	—
O.....	—	—	—	—	0	—	—	—	—	—	—	+++

\* In this table the plus signs indicate the intensity of reaction; — indicates negative reaction of some degree of agglutination, as given with normal rabbit serum; and 0 indicates reaction not done.

granular precipitate in broth and fell roughly into one group by cross fixation reactions. Cross agglutinations among members of this group, as well as among the other strains, are generally lacking, the only exceptions being noted in strains *A* 148 and *B* 4. On the other hand, cross complement fixation permits a rough classification into two larger groups, with the two strains *A* 148 and *B* 4 as connecting links. Strains *M B* and *A* 56 seem to be closely related by means of immune bodies, as do *B* 29 and *O*. Each of these three pairs is composed of members that have different fermentation reactions, indicating that immune and biochemic characteristics may be quite distinct. Furthermore, grouping according to fermentation reactions does not correspond to differences in effect on red blood cells.

In practically every way, the various organisms appear to be distinct from one another. This fact emphasizes the futility of a general or shot gun serum therapy or vaccination. If specific immune therapy is to be of avail, it must be induced by means of the homologous organism. The demonstration of immune bodies in the serum of patients seems to indicate that the patient is making an effort to combat the infection. It was thought that a third factor, such as complement, might be lacking in this disease and that transfusion of normal blood might supply this missing substance. That the hypothesis was not correct seems to be indicated by the failure to obtain any diminution of bacteriemia following transfusion unaccompanied by febrile reaction, and the fact that temporary diminution in bacteriemia occurred when febrile reactions were induced by either transfusion or injections of impure saline. These results are of striking interest in view of the recent ideas concerning nonspecific factors in the treatment of infections as expounded by Jobling and Petersen<sup>27</sup> and Miller and Lusk.<sup>28</sup> Our results show conclusively that the nonspecific febrile reaction was followed by a temporary increase in antibacterial power in the blood. The various factors concerned in the increase have not been determined.

#### CONCLUSIONS

1. Subacute streptococcus endocarditis is a disease of definite bacteriology, each case yielding a constant individual type of organism.
2. The streptococci belong to the saprophytic types according to Andrewes and Horder's classification and are of low virulence.
3. They are nonhemolytic and may or may not be green producers.
4. Biochemic and immunologic tests fail to show any constant identity between the individual streptococci concerned in producing this disease.

27. Jobling, J. W., and Petersen, W.: Jour. Am. Med. Assn., 1916, **66**, 1753.

28. Miller, J. L., and Lusk, F. B.: Jour. Am. Med. Assn., 1916, **66**, 1756.

5. The patients' blood serums contain agglutinating and complement fixing antibodies.

6. The reduction in the bacteriemia following transfusion with blood and impure saline depends on febrile reactions on the part of the patient. If such reactions do not occur, as when saline-free transfusions are used, no reduction in bacteriemia results. No noticeable benefit accrues in either case.



## BACTERIOLOGIC STUDIES IN ACUTE RHEUMATIC FEVER\*

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The present study was undertaken to determine whether any constant cultural or immunologic type of bacterium was associated with acute rheumatic fever. This disease presents a fairly typical clinical picture, consisting of an acute febrile condition, associated with polyarthritis, and a tendency to involvement of such serous surfaces as the endocardium, pericardium and pleura. Muscular tissues are also frequently involved, most noticeably the myocardium, in which a peculiar pathologic picture is commonly found in fatal cases. Certain symptoms of the disease, namely, the fever and polyarthritis, respond in a fairly characteristic manner to the administration of the salicylates in sufficient doses. It does not seem unreasonable, therefore, to suppose that the disease may be the result of the action of some definite pathogenic micro-organism.

The early contention of Achalme<sup>1</sup> that the disease was due to a gram-negative anaerobic bacillus has been disproved. The next view was that of Singer,<sup>2</sup> who claimed that the disease was an attenuated pyemia, due most frequently to the streptococci, but also to the staphylococci and other pyogenic organisms. Singer's contentions were based on bacteriologic studies of postmortem material and the isolation of pyogenic cocci from the urine. The sources of error connected with such studies are even more obvious today than they were to his opponents at that time. The extensive studies of Westphal, Wassermann and Malkoff<sup>3</sup> with a streptococcus which they isolated in a case of rheumatic fever, chorea and hyperpyrexia proved that a nonsuppurative polyarthritis could be produced in rabbits by a streptococcus. These authors attributed an etiologic rôle to the organism which they studied. Meyer<sup>4</sup> made cultures from the throats of acute rheumatic fever patients directly into broth. The injection of this

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1. Achalme, P.: *Compt. rend. Soc. de biol.*, 1891, Ninth Series, **3**, 651.

2. Singer, G.: *Aetologie u. Klinik d. acuten Gelenkrheumatismus*, Vienna and Leipzig, 1898.

3. Westphal, Wassermann and Malkoff: *Berl. klin. Wchnschr.*, 1899, **36**, 638.

4. Meyer, Fr.: *Verhandl. d. 19 Cong. f. inn. Med.*, 1901, p. 542.

mixed culture into rabbits was followed by a nonsuppurative arthritis; 10 per cent. of the animals had pericarditis or pleurisy and one fourth of the animals showed a verrucous endocarditis. From all of these lesions a diplostreptococcus which clouded broth could be isolated. The joint exudates soon became sterile and the animal recovered without permanent injury to the joint. Because cultures from normal throats, or from the angina of other disease, produced either suppurative arthritis, fatal septicemia, or no lesions at all, Meyer ascribed a specific etiologic rôle to these streptococci.

Menzer,<sup>5</sup> while confirming Meyer's results with streptococci from rheumatic angina, on the other hand was able to produce a similar arthritis and endocarditis with cultures from nonrheumatic or normal tonsils. He was unable to determine any colony, cultural or staining peculiarities in the streptococci from the tonsils of rheumatic patients compared with those from the tonsils of normal or nonrheumatic persons. He therefore concluded that "acute articular rheumatism is a peculiar clinical picture, not because there is a specific etiologic agent, but because the patient under certain inherited or acquired conditions and under acute or chronic influences reacts to the infection with common micro-organisms, apparently the organism found in the mouth, in a specific manner with manifestations which appear clinically as a specific illness."

Unfortunately, in none of these studies was any attention given to the hemolytic properties of the streptococci, so it is impossible to correlate absolutely the results with those of later workers. The evidence brought forward by Cole<sup>6</sup> and by Harris<sup>7</sup> to show that any streptococcus may produce destructive lesions of the joints likewise does not clear up this point, because the organisms studied were not divided into hemolytic and nonhemolytic forms. From our own studies and a review of the literature it is evident that the hemolytic forms may produce acute suppurative and destructive arthritis, provided they are not sufficiently virulent or injected in large enough doses to cause a fatal septicemia.

The work of Cecil<sup>8</sup> in this laboratory, however, conclusively demonstrates that destructive lesions of the articular surfaces often follow the intravenous injection of *Streptococcus viridans* into rabbits. The character of the exudate in this series was mucoid or mucopurulent. The exudate in the arthritis produced by hemolytic streptococci is frankly purulent.

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5. Menzer, A.: Die Aetologie des acuten Gelenkrheumatismus, Berlin, 1902.

6. Cole, R.: Jour. Infect. Dis., 1904, **1**, 714.

7. Harris, N.: Tr. Chicago Path. Soc., 1905, **6**, 303.

8. Cecil, R.: Jour. Exper. Med., 1916, **24**, No. 6.

In 1900 Poynton and Paine<sup>9</sup> gave the specific name "*Diplococcus rheumaticus*" to a diplostreptococcus which they isolated from four pericardial exudates, two blood cultures, two valve lesions and one angina, all from patients with acute rheumatic fever. Because they could demonstrate microscopically these organisms in rheumatic lesions, and produce similar lesions in experimental animals with pure cultures, they felt justified in applying a specific name to them. So thoroughly imbued are these authors with the specific nature of their diplococcus that they<sup>10</sup> apply the adjectives "rheumatic malignant" to the disease condition usually recognized as subacute streptococcus endocarditis. This they do because of the similarity of the micrococci associated with the two conditions. It is thus evident that their *Diplococcus rheumaticus* is similar to the *Streptococcus viridans* or *mitis*. Their cultural studies show that their organism is a diplostreptococcus giving a brownish color on blood agar plates, a turbidity in broth, and is gram-positive, though giving up the Gram stain more readily than do other gram-positive bacteria. The only point of difference from the ordinary *Streptococcus viridans* is in the size of the individual cocci, which they give as 0.5 microns. It is probable that the organisms measured by them were unusually small, such as are not infrequently encountered.

Another view of the rôle of streptococci in acute rheumatism is that of Rosenow<sup>11</sup> that the streptococci are constantly undergoing changes, assuming and discarding elective affinities for specific structures. It will be seen that this is the direct antithesis of Menzer's opinion, which places the onus on the patient's susceptibility to infection with mouth streptococci. This incomplete review of the literature gives a fair idea of the various opinions of the relation of streptococci to acute rheumatic fever.

While none of the classifications of streptococci are absolutely satisfactory, that proposed by Schottmüller<sup>12</sup> is almost universally accepted. In it we have hemolytic and nonhemolytic forms and, under the last group, those which form methemoglobin from the hemoglobin—the so-called viridans—and those which do not affect the hemoglobin, called anhemolyticus or saprophyticus by Le Blanc.<sup>13</sup>

A more extensive classification has been made by Andrewes and Horder,<sup>14</sup> based on the fermentation reactions of the streptococci.

9. Poynton, F. J., and Paine, A.: Complete works collected in Researches on Rheumatism, London, 1913, p. 109.

10. Poynton, F. J., and Paine, A.: Complete works collected in Researches on Rheumatism, 1913, p. 312.

11. Rosenow, E. C.: Jour. Am. Med. Assn., 1915, **65**, 1687.

12. Schottmüller, H.: München. med. Wehnschr., 1903, **50**, 849.

13. Le Blanc: Centralbl. f. Bakteriöl., Orig., 1911, **61**, 68.

14. Andrewes, F. W., and Horder, T. J.: Lancet, London, 1906, **2**, 708, 775, 852.



These authors recognized types which were associated to a certain extent with distribution in nature and pathogenic properties. While this classification has not been usually accepted, the study of fermentative reactions is of value and helps in a general way to place the organisms in groups.

In reviewing the various biochemical studies of streptococci, one occasionally meets with the results of studies made on organisms which have been isolated from patients with acute rheumatism. In trying to correlate these results one is struck with the diversity in types reported. Thus Andrewes and Horder<sup>14</sup> report that in the necropsies of fatal cases they secured two different main types, namely, *Streptococcus pyogenes* twice and *anginosus* variants seven times. They consider that these organisms may have been agonal invaders, because streptococci were only occasionally recovered from fatal cases. In testing a streptococcus rheumaticus from Poynton and Paine they found it to fall into the salivarius group and a *Micrococcus rheumaticus* sent by Beattie fell into the fecalis group. Beattie and Yates<sup>15</sup> report that three different streptococci isolated from the synovial membrane of the knee joint of acute rheumatic patients fell into the fecalis group. Floyd and Wohlbach<sup>16</sup> found two hemolytic streptococci, one, which clotted milk, fermented raffinose and salicin, hence fell into the *Streptococcus anginosus* group; the other did not change milk or the test sugars except lactose and fell into the pyogenes group. Still another one was nonhemolytic and fermented none of the sugars. Hopkins and Lang<sup>17</sup> found one nonhemolytic streptococcus which did not ferment any of the test objects. Lyall<sup>18</sup> reported on five streptococci, three isolated from the blood of rheumatics and two from the joints. All were indifferent to blood; one did not ferment either raffinose, inulin, salicin or mannite; one fermented only salicin, and three fermented salicin and mannite, which is characteristic of fecalis types. This small list proves that no single type of streptococcus has been constantly isolated in cases of acute rheumatic fever. Rosenow,<sup>19</sup> in reporting on the etiology of acute rheumatism, states that he was able to isolate three different types of organisms from the joint exudates. Tested on blood agar plates, they were, first, a green type forming long chains; second, a type giving slight hazy hemolysis about the colonies and forming short chains; all of Type 2 were mannite fermenters; third, a type with small grayish colonies indifferent to blood. In this paper Rosenow reported on his method of isolating

15. Beattie, J. M., and Yates, A. G.: Jour. Path. and Bacteriol., 1911, **16**, 247.

16. Floyd, C., and Wohlbach, S. B.: Jour. Med. Research, 1913, **29**, 493.

17. Hopkins, J. A., and Lang, A.: Jour. Infect. Dis., 1914, **15**, 63.

18. Lyall, H. W.: Jour. Med. Research, 1914, **30**, 487.

19. Rosenow, E. C.: Jour. Infect. Dis., 1914, **14**, 61.

the organisms from joint fluid by mixing the fluid in deep tubes of ascites dextrose agar, and from the blood by placing the sediment of centrifugalized laked blood in a similar medium. In this way streptococci were obtained from the joint fluid of fourteen out of sixteen patients; from the blood in three out of four.

We have studied the type of streptococci which we have been able to isolate from the blood of patients with acute rheumatic fever. In all fifty-eight cases have been studied. Nine were studied at the Hospital of the Rockefeller Institute three years ago and the remainder have been from the wards of the Presbyterian Hospital during the past two years. All were typical cases, showing fever and poly-arthritis, and responded in the characteristic manner to salicylates. Blood cultures were made shortly after the admission of the patients to the hospital and before salicylates had been administered; also afterward at such times as complications or relapses appeared. The cultures were made from a few hours to several days after the appearance of the arthritis. The cultures of joint fluids were made at the same time, but in many of the cases it was difficult to obtain fluid from the affected joints. For this reason there were fewer cultures of joint exudates. The joints aspirated were knee, ankle, elbow and wrist.

**Cultural Methods:** In every case blood cultures were made as follows: (1) of from 2 to 3 c.c. of blood in dextrose agar plates; (2) by placing from 5 to 10 c.c. of blood in 125 c.c. of 1 per cent. dextrose broth; (3) by laking 5 c.c. of blood in 50 c.c. of sterile distilled water, centrifugalizing and placing the sediment in deep tubes of ascites dextrose agar. The cultures were usually made in duplicate. In addition, in many instances, laked sediment was placed on the surface of blood agar slants and in ascites dextrose broth.

In making cultures of joint fluid, deep tubes of ascites dextrose agar were mixed with increasing amounts of the fluid, in most cases 1, 2, 3, and 4 c.c. of fluid were placed in the different tubes. If there was sufficient fluid it was also put into deep tubes of ascitic dextrose broth and on the surface of blood agar slants. In this way all degrees of oxygen tension were obtained. The results are summarized in Table 1.

Altogether eighty-five blood cultures were made on fifty-eight patients with seven positive results, a percentage of 8.3. In one instance the cultures 59 *F* and *B* 38 were obtained from the same patient, so that in only six patients were positive cultures obtained. With the exception of this case, positive cultures were obtained only once in each patient and repetition of the cultures a short time afterward gave negative results. In three instances the cultures were

obtained at the time of an acute pericarditis. In two of these *A* 49 and 38 *D*, adults, previous cultures had been sterile, but at the time of the appearance of the pericarditis positive cultures were obtained, while later cultures were sterile. The other case of pericarditis was that of a child, 59 *F*, admitted to the Rockefeller Hospital in 1914. The culture was positive at the time of admission, but negative later. In 1916 she was readmitted to that hospital with typical acute rheumatism and polyarthritis; culture *B* 38 was obtained, but repeated attempts to secure a confirmation resulted in sterile cultures. Culture *B* 39 was from a child and *A* 65 and *A* 135 from adults with typical acute rheumatic fever.

All of the cultures of joint exudates have been sterile. Joints have been aspirated within four hours after the appearance of the exudate and at various later intervals. As positive blood cultures have been obtained from patients at the time that joint exudate cul-

TABLE 1.—RESULTS OF BLOOD AND JOINT CULTURES

	Blood Cultures			Joint Cultures		
	Number of Patients	Number of Cultures	Number of Positive Cultures	Number of Patients	Number of Cultures	Number of Positive Cultures
Adults.....	44	60	4	23	31	0
Children.....	14	25	3	2	3	0
Total.....	58	85	7 (8.3%)	25	34	0

tures were sterile, it is evident that the medium was suitable for the growth of the streptococci. We therefore do not feel that the difference between our results and those of Rosenow can be explained by technical errors, such as age of the arthritis or faults with culture mediums. In addition all of the joint exudates have been examined microscopically and in no instance have we found diplococci or streptococci in smears.

Patients have also been studied in whom arthritis was not present. These include principally cases of endocarditis with fever and accompanying conditions, such as pleurisy, pericarditis or chorea. While it is generally considered that these are rheumatic manifestations, it was thought better to place them under a separate heading. In two such instances streptococci were obtained in blood culture. Culture *A* 119 was obtained from a patient during a febrile attack accompanied by pain in the knee, but no other arthritis symptoms. Culture *A* 141 was obtained from a child with pericarditis and endocarditis during an attack of "decompensation" and pleurisy. Subsequent cultures were



sterile, but the "rheumatic" nature of the condition was demonstrated a year later at necropsy. In addition, three cases of acute rheumatic pericarditis and two of endocarditis gave sterile blood cultures during the acute period of the disease. The aspirated pericardial fluid from one of the pericarditis patients also yielded negative cultures.

Necropsies: The difficulty of obtaining a pure culture at necropsy is almost insurmountable and hence the results of necropsy cultures are inconclusive. Six fatal cases have been studied. Three of these patients had acute pericarditis and acute endocarditis, two acute endocarditis and one chorea and endocarditis with terminal pneumonia.

TABLE 2.—RÉSUMÉ OF POSITIVE CULTURES

Number	Day of Disease after Onset of		Mediums in Which Positive Cultures Grew*	Joint Cultures
	Arthritis	Pericarditis		
A 65	4th	0	Laked sediment in ascites dextrose agar tube only	Sterile
A 135	11th	0	All mediums.....	Sterile
B 39	4th	0	Two broth flasks, no others made.....	Not made
59 F	1st attack 9th	1st	One broth flask only.....	Not made
B 88	2d attack 2d	....	All mediums; 100 colonies per c.c. ....	Sterile
38 D	12th	....	Sterile	
	15th	1st	Laked sediment in ascitic broth only....	Sterile
A 49	14th	....	Sterile	
	19th	1st	Colonies in only 1 dextrose blood agar plate	Not made
A 119	Indeterminate 2d day of febrile attack	Previous attack	Colonies in only 1 dextrose blood agar plate	Not made
A 141	Indeterminate during pleurisy	Previous attack	Laked sediment in 1 ascitic dextrose agar tube only	Not made
A 179	Necropsy	2½ mo.	Blood agar plates	

\* Positive cultures developed in all mediums in two cases; laked sediment in deep ascitic dextrose agar tubes only, in two cases; laked sediment in ascitic broth only, one case; blood broth flask only, one case; both blood broth flasks, one case; dextrose blood agar plates only, two cases.

From the vegetations of one of the endocarditis cases streptococci were grown in pure culture and from the other a mixed culture of staphylococci and diphtheroid bacilli. From the heart's blood of the chorea and endocarditis patient, a Group 4 pneumococcus was obtained. In the cases of pericarditis and endocarditis the cultures from endocardial lesions in one case yielded a staphylococcus and a green-forming streptococcus which fermented raffinose. From the second case, in which the heart was opened under rigid precaution after removal from the necropsy room, three types of cocci were

obtained, (1) pneumococcus, (2) hemolytic streptococcus, and (3) a green-forming streptococcus. The streptococci from both these cases were lost before they were completely studied. In the third case A 179, diphtheroid bacilli and green streptococci were obtained from all the diseased valves. The characteristics of these cocci are given under the subsequent studies. The case reports are given at the end of the paper.

*Cultural Characteristics.*—The cultural characteristics and virulence of the organisms have been studied immediately on isolation. All were gram-positive cocci, with a tendency to grow in pairs or short chains. Plain broth cultures were turbid and did not clear on the

TABLE 3.—CULTURAL AND VIRULENCE CHARACTERISTICS

No.	Growth in		Bile Solu- bility	Effect on Red Blood Cells	Fermentation Reactions					Lethal Dose for White Mice
	Bouil- lon	Aseptic Dex- trose Agar			Milk	Raffi- nose	Inu- lin	Sali- cin	Man- nite	
B 38	Turbid	Marked clouding	Insoluble	Green	+	+	+	0	0	2 c.c. 0
A 179	Turbid	Marked clouding	Insoluble	Green	+	0	0	0	0	2 c.c. +
B 39	Turbid	Marked clouding	Insoluble	Green	+	0	0	0	0	2 c.c. 0
59 F	Turbid	Marked clouding	Insoluble	Green	+	0	0	0	0	0.1 c.c. +
A 49	Turbid	Marked clouding	Insoluble	Green	+	0	0	0	0	0.1 c.c. +
A 141	Turbid	Marked clouding	Insoluble	Green	+	0	0	0	0	2 c.c. +
A 119	Slightly turbid	Marked clouding	Insoluble	Slightly green	+	0	+	+	0	2 c.c. 0
28 D	Slightly turbid	Marked clouding	Insoluble	Green	+	0	0	+	0	1 c.c. +
A 135	Turbid	Marked clouding	Insoluble	Green	+	0	0	+	+	2 c.c. 0
A 65	Turbid	Marked clouding	Insoluble	Green	+	+	0	0	0	0.1 c.c. +

addition of equal parts of ox bile. No capsules could be demonstrated in the smears of the peritoneal exudates of mice inoculated intra-peritoneally with the organism. The action on blood was studied, both on human blood plain agar plates and by mixing diminishing dilutions of twenty-four hour plain broth cultures with equal parts of 5 per cent. suspension of sheep's blood cells and incubating two hours. None of the strains produced hemolysis of the cells or clearing about the colonies on the blood agar plate and all turned the cells brown and gave a green appearance on the plates. This is characteristic of the so-called *Streptococcus viridans*.

The fermentation reactions were studied with milk, raffinose, inulin, salicin and mannite in Hiss serum water mediums. These mediums were heavily inoculated with an actively growing twenty-four-hour broth culture, and observed over a period of ten days. The fermentation reactions were studied at the time of isolation of the culture, and again in June, 1916. None of this series underwent any change during the interval. All the strains produced acid and clot in milk. Five of the strains did not ferment any of the test objects. The other five showed individual fermentative capacity, no two falling into the same group. Only one was a mannite fermenter, thus placing only one of our series in the fecalis group.

The virulence was tested by injecting 2 c.c. 1 c.c. and 0.1 c.c. of a twenty-four-hour plain broth culture intraperitoneally into white mice. The lethal dose of three of the strains was 0.1 c.c., of one it was 1 c.c., of two it was 2 c.c., and four were not fatal in 2 c.c. quantities. Only in the point of virulence have organisms in this group shown peculiarities. Compared with green-producing streptococci from normal mouths or from patients with subacute bacterial endocarditis most of these strains have been distinctly virulent, although compared with hemolytic streptococci or pneumococci, their virulence is low. On cultivation the original virulence has been fairly well maintained, and repeated passage through mice has failed to increase the virulence to any appreciable degree.

*Immunologic Classification.*—In addition to a study of the cultural characteristics, an attempt has been made to determine whether there was any immunologic grouping. For this purpose the complement fixation reaction has been used, employing antiformin solutions of the various streptococci as antigens. The exact method is fully described elsewhere.<sup>20</sup> Rabbits were injected intravenously with killed streptococci until their serum gave good fixation with its homologous antigen. It was then tested for its complement fixing power against antigens prepared from all the streptococci. The results are summarized in Table 4.

It will be seen that no single group exists. One member, *A* 65, is not related to any of the others. Among the remaining strains roughly three groups can be made out. The first consists of three strains, *B* 38, *A* 179 and *B* 39; the second of 59 *F* and *A* 49, which appear to be almost identical; and a third, which consists of *A* 141, *A* 119 and 38 *D*. The first and second groups are connected by serum *A* 141, which also fixes antigen *A* 135. Comparing the individual strains, however,

20. Kinsella, R. A., and Swift, H. F.: A Classification of Nonhemolytic Streptococci. To be published.



we find that the only two which appear to be very closely related are 59 F and A 49.

It is not the intention to discuss the general subject of the classification of streptococci by means of the complement fixation reaction, as that is more fully considered in another communication. The evidence brought forward in this table is merely to show the immunologic relationship of the various strains.

A comparison of the fixing properties with the fermentation reactions and virulence of the several strains shows that no strict relationship exists between the various fermentation groups and the groups as determined by complement fixation. In the study of the virulence of the strains no special tendency toward virulence is noted among any one of the various fermentation groups or complement fixation groups,

TABLE 4.—GROUPING BY MEANS OF COMPLEMENT FIXATION REACTIONS \*

Serum of Rabbits Immunized With	Antigens									
	B 38	A 179	B 39	59 F	A 49	A 141	A 119	38 D	A 135	A 65
B 38.....	+	—	±	—	—	—	—	—	—	—
A 179.....	—	+	±	—	—	—	—	—	—	—
B 39.....	+	+	+	+	±	—	—	—	—	—
59 F.....	—	—	—	+	+	—	—	—	—	—
A 49.....	±	±	—	+	+	—	—	—	—	—
A 141.....	—	—	—	+	+	+	+	+	+	—
A 119.....	—	—	—	—	—	—	+	+	—	—
38 D.....	—	—	—	—	—	—	—	+	—	—
A 135.....	—	—	—	—	—	—	—	—	+	—
A 65.....	—	—	—	—	—	—	—	—	—	+

\* In this table + signifies that fixation with heterologous antigen was of practically the same strength as with homologous antigen; ± signifies that fixation with heterologous antigen was weaker than with homologous antigen; — signifies negative reaction.

with the exception of 59 F and A 49, which seem to be closely related in all their characteristics. Compared with a larger number of green-forming streptococci obtained from blood cultures of *Streptococcus viridans* from endocarditis patients and from the throats of acute angina patients, the streptococci from acute rheumatic fever patients could not be differentiated by means of cultural characteristics. In the immunologic studies most of the streptococci from rheumatic fever patients fell in one side of the large group, although various individual members are scattered throughout the group. In the matter of virulence six out of ten rheumatic fever strains were virulent, while with one exception all the endocarditis strains were avirulent. On the other hand, angina strains were often virulent to 1 c.c. or less.

*Study of Antibodies in Patients' Serum.*—After it was shown<sup>21</sup> that the serum of patients with subacute streptococcus endocarditis contained antibodies against homologous strains, it was thought that antibodies might be present in the serum of acute rheumatic fever patients whose blood yielded a positive culture. Likewise, if a specific "*Streptococcus rheumaticus*" existed, the serums of rheumatic fever patients might show specific antibodies. These antibodies were tested for by means of the thread reaction, a modification of the agglutination reaction which has proved more satisfactory with streptococci than the ordinary agglutination reaction. The serums of five patients tested failed to react to their respective streptococci. The serums of twelve other rheumatic fever patients failed to react with any of the four strains, A 49, 59 F, 38 D and A 65, a result which is easily explained by the individuality of these strains. The failure of serums to react with homologous strains is, however, most striking when compared with the results obtained in a type of disease such as subacute streptococcus endocarditis, in which we are certain of the relationship between the bacterium and the disease.

#### COMMENT

We do not feel that our results clear up the much-vexed question of the relation of the nonhemolytic streptococcus to acute rheumatic fever. From a statistical point of view the small percentage of positive cultures would incline toward the negative side of the question. Streptococci are found in the blood of scarlet fever patients with an equal frequency, and yet most observers do not believe that the streptococcus is the cause of scarlet fever, but rather that this organism acts as a secondary invader; and it is conceivable that it may play a similar rôle in acute rheumatic fever. The relatively high frequency with which the streptococcus was demonstrated in patients with complications such as pericarditis or pleurisy lends support to this opinion. Again, the failure to demonstrate constantly the streptococcus in the verrucae of acute rheumatic endocarditis is an argument against the specific etiologic relationship of this organism.

On the other hand, a small proportion of positive cultures may simply mean that the streptococci circulate in the blood stream only for short periods, or are rapidly killed off, so that the methods employed are sufficient to demonstrate them only occasionally. Furthermore, our studies of the cultural and immunologic characteristics show that the streptococci which are present in this disease are merely various members of the *Streptococcus viridans* group, and should not be separately classified as *Streptococcus rheumaticus*. Of extremely

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21. Kinsella, R. A.: THE ARCHIVES INT. MED., this issue, p. 367.

important bearing on this question are the findings in cultures 59 *F* and *B* 38. At the time of the first attack, streptococcus 59 *F* was isolated. After a prolonged course the patient recovered from every evidence of acute or subacute infection. After a year of good health she suffered a relapse, or as is more probable, a new infection. At this time streptococcus *B* 38 was recovered from her blood. This proved to be an organism that differed culturally, immunologically and in its virulence from the strain originally isolated. In addition, no complement fixing or agglutinating antibodies against 59 *F* could be demonstrated in the patient's blood at the time of the second infection. If the infection was a chronic one due to streptococcus 59 *F*, we would expect to find antibodies such as may be demonstrated in the serum from cases of subacute streptococcus endocarditis. We feel that more detailed observation and experimentation is required to decide the points at present under discussion.

Another point should be mentioned: There is a tendency among some workers to consider that because a green streptococcus is isolated from the blood of a patient with endocarditis, there is sufficient ground for classifying the case in question as subacute bacterial endocarditis—the type so well described by Libman. This disease, with its characteristic symptomatology, is now generally recognized as a fatal malady. From our studies we feel that the essential factors in its recognition do not depend alone on the isolation of the infecting organism, but on such characteristic symptoms and signs as petechiae, embolic lesions and progressive downward course, in addition to the picture of an endocarditis. The findings in our cases indicate that green streptococci can be isolated from the blood stream, and still the patient may not suffer from subacute bacterial endocarditis. Some of the patients have been followed for a year or more without showing any signs of this disease, and case *A* 141, a year after yielding a single positive blood culture, showed at necropsy characteristic rheumatic lesions in the heart valves and muscle.

#### SUMMARY AND CONCLUSIONS

1. Cultures of the exudate aspirated from the joints in acute rheumatic arthritis have been uniformly sterile.
2. Nonhemolytic streptococci have been recovered in blood culture from less than 10 per cent. of patients suffering from acute rheumatic fever.
3. Similar streptococci have been recovered from the active endocardial lesions in only half of the fatal cases of acute rheumatic fever.
4. From the above results it seems evident that no type of streptococcus has been constantly associated with acute rheumatic fever.



5. We do not feel that the etiologic relationship between the streptococcus and acute rheumatic fever has been definitely proved, but if the streptococcus is the etiologic agent in acute rheumatic fever, it is shown by means of cultural and immunologic studies that it is through various members of the viridans group, and hence no one member can be called the *Streptococcus rheumaticus*.

#### CASE REPORTS

*A 65*.—J. C., a man aged 19, a painter, was admitted to the Presbyterian Hospital Jan. 8, 1915, and discharged February 7. On January 9, herniotomy was performed. The temperature was normal four days and then it ranged between 99 and 103 for seven days. On the 19th blood cultures made in broth flasks were sterile. On the 25th the patient suffered pain in both ankles and the right knee. On the 27th there were general malaise and chilly sensations, the joints being swollen and tender. Blood culture in broth flasks was sterile. On the 28th blood cultures in broth flasks and ascitic dextrose agar plates were sterile. On the 29th blood cultures were positive for *A 65*. Cultures were developed only in laked sediment in ascitic dextrose agar tube. A culture of fluid from the right knee was sterile. On the 31st extension of arthritis to wrist and hands followed. Prompt relief was obtained from 100 grains of sodium salicylate per day. On February 7 the patient insisted on going home because he felt so well. The serum gave no thread reaction with *A 65* culture.

*A 135*.—O. C., a man aged 26, a tile-layer, was admitted to the Presbyterian Hospital April 7, 1915, and discharged on May 11. He had suffered six years before with "acute rheumatism," involving all the joints. He was ill four months five years before with a similar attack, his illness lasting one month. His present illness began two weeks before with "cold in head and chest." Eleven days before his left knee became swollen, tender and painful; this was followed by the involvement of all the joints of the arms and hands and the right knee. There was very little general illness. Physical examination revealed pyorrhea. There was an abscess at the root of the first right lower molar. The heart was not enlarged, but there was a faint murmur at the apex not transmitted and at the base. There was general polyarthritis. On April 7 a blood culture proved positive for *A 135*. Growth took place in all mediums. The right knee yielded 20 c.c. of murky, lemon-colored fluid, the left 15 c.c. of similar fluid. Cultures of this fluid were sterile. Fever was absent after three days of salicylates, and the joints returned to normal after five days. The patient was discharged without any complications of the disease.

*B 39*.—B. M., a girl, aged 6 years, was admitted to the Presbyterian Hospital March 10, 1916, and discharged April 9. The family history showed that the mother and one sister had syphilis. The child had had some joint trouble when a few weeks old. She had acute rheumatism eighteen months before admission and numerous attacks of tonsillitis, the last being one month before. Three days before there was pain in both wrists, lasting two days, and one day before the ankles became painful and swollen. Physical examination showed the tonsils much enlarged, not acutely inflamed. The heart was enlarged to the left and downward. There was a systolic murmur at the apex. A diastolic murmur was perceptible over the aortic area. Both ankles were hot, tender and swollen. On March 11 a blood culture in two flasks showed both positive for *B 39*. On the 14th a blood culture proved sterile. The arthritis cleared up without salicylates. On April 7 a blood culture was sterile and the general condition was good.

*59 F and B 38*.—F. B., a schoolgirl, aged 8 years, was admitted to the Rockefeller Institute Hospital May 26, 1914, and discharged June 2, 1915. The child was suffering with rheumatic fever, acute pericarditis, valvular disturbance and mitral insufficiency. She complained of pain over the heart. Two weeks previously the mother and one brother of the patient had sore throat, but the patient did

not. The patient had measles at 4, but no scarlet fever. She often had bronchitis, but no sore throat. At 1 year there was twitching of the left arm (chorea). She never had pain in the joints. Eleven days previously the patient fell while skating. Ten days before she vomited and had headaches. Nine days before her feet hurt and the right one was swollen. Eight days before both ankles and knees were swollen and painful. This gradually decreased. Her general condition improved from day to day. The day before entrance she was out of bed, and on the day of entrance she was attacked with a sudden pain in the region of the heart. Physical examination revealed dyspnea, painful expression and a dry cough. There was a friction rub over heart. The heart was 4 cm. to the right and 11 to the left of the midsternal line. The liver was enlarged, extended 3 cm. below the costal margin and was tender. There was no edema. The joints were negative. Blood culture was positive for 59 *F*. Growth took place only in the broth flask. The friction rub persisted until June 8. Fever lasted until July 12. From September 21 to 29 there was fever, but the blood cultures were sterile. On Aug. 7, 1915, the tonsils were removed.

The patient was readmitted to the hospital March 9, 1916. She had remained well until one month before, when she had grip and fever for three days. Three weeks before she had grip for two weeks, with high fever; then she was well one week. Two days before entrance she vomited and had headache. The day before she had pain in both ankles. Examination showed no petechiae. The throat was clear. The heart was enlarged, and there was an apical systolic murmur. There was heat and tenderness in the left ankle. A blood culture was positive for *B* 38, giving 100 colonies per cubic centimeter, which developed in all mediums. February 12 both knees were swollen and tender. March 10 the ankles, knees, elbows and wrists had been involved. Blood cultures on March 11, 12 and 13 were sterile. On the 12th 5 c.c. turbid fluid were aspirated from the knee joint, but a culture proved sterile. There was no trace of petechiae. The fever ranged from 101 to 102 until May 5.

38 *D*.—J. McK., a blacksmith, aged 58, was admitted to the Rockefeller Institute Hospital March 15, 1914, and discharged June 9, 1914. There was a history of acute rheumatism four years previously. There had been no cardiac symptoms afterward. Two weeks before entrance he caught cold, and eleven days before his left wrist became red and swollen. Five days before his knees became involved, and four days before his right wrist also was affected. He was confined to bed for two days. Examination showed the heart to be slightly enlarged. There was an apical systolic murmur. There was acute arthritis in the right wrist, both elbows, the knees and the ankles. On March 16 a blood culture was sterile, and the fluid from both knees and ankles was sterile. On March 19 there was a pericardial friction rub. A blood culture was positive for 38 *D*, developing from laked sediment in ascitic broth. On March 20 a blood culture was sterile. On the 21st there were signs of fluid in the left pleural cavity. On the 26th the pericardial rub had disappeared and the blood culture was sterile. From March 29 to April 11 there was continuous auricular fibrillation. On April 12 the heart showed sequential rhythm. May 9 the patient showed a slow but steady convalescence.

A 49.—A. G., a waitress, aged 23, was admitted to the Presbyterian Hospital Jan. 16, 1915, and discharged April 6, 1915. Eleven years before the patient had a severe acute articular rheumatism, followed by chorea. The present illness began two weeks before with polyarthritis, involving both arms and legs. Later this had receded, but there were fever and sweating, and the previous few days dyspnea. Examination showed the heart to be enlarged, and there was an apical systolic murmur. The left wrist was swollen, red and tender. On January 16 blood culture proved sterile. From the 17th to the 19th the wrists, shoulder and knees were involved. On January 21 there was a pericardial rub.<sup>22</sup> Blood

22. During the period from Jan. 16 to 23, 1915, the patient was taking sodium salicylate from 100 to 200 grains per day.



culture was positive for *A* 49, colonies arising in one blood agar plate. This was followed by effusion into the pericardium, which persisted until the middle of February. On February 1 she had a febrile attack without new symptoms, but a blood culture was sterile. On the 3d there was a fresh effusion into the knee joint, but a culture was sterile. Convalescence was slow but steady until the patient was discharged April 1.

In the spring of 1916 the patient reported another attack of acute rheumatic fever without cardiac involvement. In May, 1916, the blood serum gave no complement fixation with antigen *A*49.

*A* 119.—H. F., a boy, aged 16, a clerk by occupation, was admitted to the Presbyterian Hospital March 2, 1915, and discharged April 9, 1915. The boy had attacks of precordial pain and dyspnea in 1909, 1910, 1911 and 1912. In 1912 he had some joint pains. In 1913 in this hospital a diagnosis was made of acute simple endocarditis, fibrinous pericarditis and left hydrothorax. There were double murmurs in both mitral and aortic areas. The boy was well and working until two weeks before entrance. He had cough two weeks, with increasing dyspnea, swelling of abdomen and legs. A physical examination showed dyspnea, orthopnea, with the throat clear, except for enlarged tonsils. The heart was much enlarged to the right and left. There were double mitral and double aortic murmurs, congestion of the lungs, enlarged liver, ascites and edema of the legs. On March 5 the patient had an attack of pulmonary edema. On the 9th steady improvement was apparent. March 14 to 16 the temperature was 102. There were precordial pain and pain in the left knee, but no swelling or redness. On March 16 a blood culture was positive for *A* 119, colonies developing on blood agar plate. There was immediate improvement after taking salicylates. April 5 the temperature was normal, and the patient showed steady improvement. A blood culture was then sterile. The boy was discharged April 9, and he returned to work.

*A* 141.—J. K., a schoolgirl, aged 10 years, was admitted to the Presbyterian Hospital Oct. 3, 1914, remaining till Dec. 29, 1914. She was readmitted April 7, 1915, and remained till June 16, 1915. She had diphtheria at the age of 5 years. She was always subject to sore throat. In April, 1914, she suffered from dizziness and vomiting. A diagnosis of rheumatic heart was made. This cleared up with rest. On Oct. 3, 1914, there were dizziness, cyanosis and dyspnea for three days. Examination showed the heart to be enlarged. An apical systolic murmur was transmitted over the entire chest. There was congestion of the lungs and enlarged liver. Fever of 103 was present, with respiration 56. On October 7 a blood culture showed three plates sterile. On the 12th a blood culture of two plates of dextrose blood agar was sterile. From the 14th to the 24th a precordial friction rub was perceptible. On the 29th there was general edema. November 24 the patient showed steady improvement, but there was fever of 101 each day. A blood culture on four dextrose blood agar plates was sterile. On December 22 the patient was much improved and was discharged.

She was readmitted April 7, 1915, complaining of increasing anorexia, nausea, pain in the chest and coughing. The throat was clear. The heart was enlarged, and showed a systolic retraction over the precordium. There were systolic and presystolic murmurs at the apex. The lungs were congested and a friction rub over the right base posteriorly was discernible. On April 13 a blood culture was positive for *A* 141, colonies developing only in laked sediment in ascites dextrose agar tube. On April 19 and May 20 blood cultures were sterile. June 1 the patient showed a steady convalescence, but she had a temperature of 101 nearly every day. On June 16, 1916, she was readmitted in extremis. A blood culture was sterile.

Necropsy showed adherent pericardium, acute vegetative endocarditis of verrucous type, chronic cardiac valvular disease, chronic mural endocarditis, adherent pericardium, pleuritic adhesions (both sides) and rheumatic myocarditis.

*A* 179.—A. B., a schoolgirl, aged 12 years, was admitted to the Presbyterian Hospital Feb. 26, 1915, and died May 20, 1915. She had acute rheumatism four



years before, lasting three weeks. Two weeks before entrance there was pain in the legs; then the shoulders, ankles and knees became involved. There was pain over the precordium for several days, increasing dyspnea and weakness. Examination showed the pharynx to be diffusely reddened and the tonsils large. The heart was enlarged to the left and downward. There was a distinct gallop rhythm, with systolic and diastolic murmurs at the apex. The liver was enlarged, and there was tenderness over it. The right hand, left ankle and right knee were tender, painful and swollen. February 27 a blood culture was sterile. On March 3 the area of cardiac flatness was much increased. The cardiohepatic angle was obtuse and the heart sounds muffled. From March 17 to April 12 the patient had attacks of slowing of the pulse in which the rate was one-half the ordinary rate. Electrocardiograms showed sudden alternating transition of auricular rate from 53 to 108. At both rates the conduction mechanism was normal. From April 14 to 18 there were precordial friction rubs, and also from May 4 to 7. On May 14 there was much precordial pain, with chorieform movements. May 20 the condition steadily grew worse until death.

Necropsy showed acute rheumatic endocarditis, involving the mitral, aortic and tricuspid valves and foramen ovale; acute pericarditis, adherent pericardium; rheumatic myocarditis; many Aschoff bodies; cardiac hypertrophy; rheumatic pleurisy; mediastinitis; petechial hemorrhages into the fatty tissue of the abdomen, the pectoralis minor muscles and the pericardium, and beneath the endocardium in the right auricle; acute hemorrhagic enteritis. Cultures showed the hemorrhagic area from the left pectoralis minor muscle to be sterile. The heart's blood showed three diphtheroid colonies. The valve lesions were cultured and diphtheroid and green forming streptococci A 179 developed from each valve, including the tricuspid valve, the mitral valve and the aortic valve.

## THE MALONE-KIUTSI REACTIONS IN PREGNANCY AND 'CANCER \*

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Various attempts have been made to simplify the technic of the Abderhalden dialysis test, but without success. Some of the investigators have applied the technic originally described by him with the use of the urine instead of the blood serum. The results have been variable.

The theoretical basis for the use of the urine has been on the supposition that the enzymes which dialyze through a parchment thimble pass through the kidney and are found in the urine (Berman,<sup>1</sup> Malone<sup>2</sup>).

L. M. Warfield<sup>3</sup> had reported seventeen analyses (Abderhalden method) on urines of pregnant women in which he had obtained positive reactions in all. Fischer<sup>4</sup> pointed out later that Warfield<sup>3</sup> had omitted the urine of males and nonpregnant females as controls. Fischer<sup>4</sup> found that in his laboratory all urines with few exceptions gave a positive ninhydrin reaction. These findings coincide with my observations. He felt that the negative reactions were due to some ninhydrin inhibiting substances, but he did not attempt to explain their nature.

Berman<sup>1</sup> had reported the dialysis test on ninety-seven urines of pregnancy, forty-eight specimens of gynecologic patients and ten of males. His positive reactions to ninhydrin after incubation were 92, 89, and 70 per cent., respectively.

Falls and Welker<sup>5</sup> in a detailed report stated that amino-acids form an appreciable part of the normal nitrogenous elimination in the urine. They reasoned that if ninhydrin reacted with compounds containing the intact amino group, then all normal urines should react positively with the reagent unless the content of the amino-acids fell below the limits of the delicacy of the reaction.

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\* From the Physiological Chemical Laboratory, Beth Israel Hospital.

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1. Berman, L.: Application of the Ninhydrin Reaction to the Urines and Urinary Dialysates of Pregnant Women, *Am. Jour. Obst.*, 1914, **70**, 192.

2. Malone, R. H.: *Jour. Am. Med. Assn.*, 1915, **64**, 1651.

3. Warfield, L. M.: *Jour. Am. Med. Assn.*, 1914, **62**, 436.

4. Fischer, C. E. M.: *Jour. Am. Med. Assn.*, 1914, **62**, 950.

5. Falls, F. H., and Welker, W. H.: *Jour. Am. Med. Assn.*, 1914, **62**, 1800.

In June, 1914, M. Kiutsi<sup>6</sup> of Japan claimed to have discovered a new modification of the Abderhalden reaction in which he had avoided using dialyzing thimbles, with excellent results. In pregnancy he had "repeated this method hundred times and never missed." Moreover, he stated in a later communication<sup>7</sup> that "it is not at all difficult with the improved method to diagnose the pregnancy in, but two hours after the test began."

He claimed that he could diagnose cancer, tuberculosis, gallbladder disease, appendicitis, retinitis, and choroiditis, and had isolated a ferment for each disease. In other words, a large number of our present common diseases could be diagnosed by laboratory methods alone.

One is impressed in a perusal of his reports by the secrecy of the details of the technic in such an important discovery. In his test for pregnancy he made use of a preparation, called by him "ninserin," as the substrate, but he did not give the details of the preparation.

In view of Malone's results in pregnancy, I made attempts to corroborate his findings and extend the usefulness of the test to cancer. This study covers sixty-seven analyses, including urines of pregnant and nonpregnant women, urines of cancer patients and urines from normal males. The results are discussed in the accompanying tables.

I undertook to determine the following:

1. The preparation of the substrate.
2. The preparation of the urine.
3. The technic of the test.
4. The determination of the period of incubation (Table 1).
5. The value of the test in pregnancy (Table 2).
6. The value of the test in cancer (Table 3).
7. The reaction of the urine of pregnant and cancer patients on both placental and cancer substrates (Tables 3 and 4).

1. Preparation of the Substrates: For the pregnancy test fresh placenta was used, while for the cancer test an adenocarcinoma of the liver was employed. The preparation in both instances was alike.

A fresh placenta was placed in normal saline solution and as much blood forced out of it as possible. It was then divided into small pieces with an ordinary meat chopper, after discarding all the blood vessels and connective tissue, and placed into running tap water for approximately thirty-six hours. The residual tissue appeared snow white. All red pieces and blood clots were removed before boiling in distilled water. Five drops of dilute acetic acid were then added to

6. Kiutsi, M.: Kiutsi's Urindiagnosis by Means of "Filtration Process," June 10, 1914, printed by Bunyeido, Sapporo, Japan.

7. Kiutsi, M.: Kiutsi's Isolation of Protective Ferments and Progress of Filtration Process, Oct. 1, 1914, printed by Bunyeido, Sapporo, Japan.



the water, as recommended by Abderhalden. It was boiled for five minutes and then washed with distilled water four or five times by shaking with water equal to five times the amount of substrate used; the supernatant fluid was decanted each time. The boiling process was repeated till 5 c.c. of filtered decanted water gave a negative reaction with 1 c.c. of 0.1 per cent. ninhydrin in solution (acetic acid was added only in the first boiling).

The next step consisted in drying the substrate in a water chamber oven at 80 C. for four hours and over night at 55 C., and then in a desiccator over calcium chlorid for three days. The resulting brown powder was very dry and hard, and was kept in a ground glass stoppered bottle in a dark place. The substrate used in the cancer tests was prepared in the same way.

TABLE 1.—DETERMINATION OF THE PERIOD OF INCUBATION \*

Specimen Number	C.c. Each Test	2 Hrs.		6 Hrs.		12 Hrs.		16 Hrs.		24 Hrs.	
		B.	N.	B.	N.	B.	N.	B.	N.	B.	N.
A 1	5	0	+	0	+	0	++	0	++	0	++
C 2	5	0	+	0	+	0	+	0	++	+	++
C 3	5	0	+	0	+	0	+	+	++	++	++
A 4	5	0	+	0	++	0	++	0	++	++	++
A 5	5	0	+	0	+	0	++	0	++	++	++
C 6	10	0	+	0	+	0	++	0	++	++	++
C 7	10	0	+	0	+	0	+	0	++	++	++
C 8	5	0	+	0	++	++	++	++	++	++	++
A 11	10	0	+	0	+	0	+	0	+	++	++
A 12	5	0	+	0	+	0	+	0	+	++	++

\* In this and the following tables B, signifies biuret test; 0, negative; + positive; N, ninhydrin test; C, pregnancy urines; and A, the urine from males. All urines were incubated at 37.5 C.

2. Preparation of the Urine: The urines were obtained immediately after voiding and examined for albumin by the biuret test. If positive, they were made negative by the following method: Fifteen cubic centimeters of urine were placed in a well-stoppered bottle and shaken for ten minutes with 0.3 gm. kaolin; it was then filtered, and tested again. The shaking process was repeated till the biuret test was negative. Attempts to shake the urine with animal charcoal obtained from several different sources were not satisfactory, inasmuch as it was necessary to keep increasing the amount of urine used and a longer time was consumed to render the urine negative. Ten c.c. of the negative urine was neutralized to litmus with 2 per cent. sodium carbonate solution, if acid, or with 1 per cent. acetic acid, if alkaline.

3. The Technic of the Test: Ten c.c. prepared urine and 0.2 gm. of

TABLE 2.—THE VALUE OF THE TEST IN PREGNANCY \*

Specimen Number	Diagnosis	C.e. Each Test	Hours Incubation	Control (1) Urine		Urine and Placenta		Control (2) Urine	
				B.	N.	B.	N.	B.	N.
1	Male—pneumonia...	5	12	0	+	0	++	0	+
2	Pregnancy.....	5	16	0	++	0	++	0	++
3	Pregnancy.....	5	16	0	+	+	++	+	++
4	Male—nephritis.....	5	12	0	++	0	++	0	++
5	Male—carditis.....	5	12	0	++	0	++	0	++
6	Pregnancy.....	5	12	0	+	0	++	0	++
7	Pregnancy.....	5	12	0	++	0	++	0	++
8	Pregnancy.....	5	12	0	++	++	++	0	++
9	Pregnancy.....	5	16	0	+	0	+	+	++
10	Pregnancy.....	5	16	0	+	0	+	+	++
11	Male—nephritis.....	10	16	0	+	0	++	+	++
12	Male—nephritis.....	10	16	0	+	0	+	0	+
13	Pregnancy.....	10	16	0	+	0	+	++	++
14	Pregnancy.....	10	16	0	++	++	+	0	++
15	Pregnancy.....	10	16	0	+	+	++	0	++
16	Pregnancy.....	10	16	0	+	0	++	0	++
17	Pregnancy.....	10	16	0	+	0	+	+	+
18	Pregnancy.....	10	16	0	+	+	++	0	+
19	Male—pneumonia...	10	16	0	+	0	+	+	++
20	Pregnancy.....	10	16	0	+	0	+	+	++
21	Pregnancy.....	10	18	0	+	0	++	0	++
22	Pregnancy.....	10	18	0	+	0	++	++	++
23	Pregnancy.....	5	24	0	+	++	++	+	++
24	Pregnancy.....	5	24	0	+	+	++	+	++
25	Pregnancy.....	10	24	0	+	+	++	++	++
26	Male—healthy.....	10	24	0	+	+	++	+	++
27	Pregnancy.....	10	24	0	+	0	+	+	++
28	Pregnancy.....	10	24	0	+	+	++	+	++
29	Pregnancy.....	10	18	0	+	0	+	0	+
30	Pregnancy.....	10	18	0	+	+	+	+	+
31	Pregnancy.....	10	18	0	++	+	++	0	++
32	Pregnancy.....	10	18	0	+	+	++	++	++
33	Pregnancy.....	10	18	0	+	0	+	+	++
34	Male—healthy.....	5	18	0	+	0	+	+	+
35	Female—Ca. breast; not pregnant.....	5	18	0	+	0	++	+	++

\* In addition to the symbols explained in the footnote to Table 1, in this and the following tables Ca. signifies carcinoma; (1), before incubation; (2), after incubation.

TABLE 2.—THE VALUE OF THE TEST IN PREGNANCY\*—(Continued)

Specimen Number	Diagnosis	C.c. Each Test	Hours Incubation	Control (1) Urine		Urine and Placenta		Control (2) Urine	
				B.	N.	B.	N.	B.	N.
36	Male—healthy.....	10	18	0	+	0	+	+	++
37	Pregnancy.....	10	16	0	+	+	++	0	++
38	Male—healthy.....	5	16	0	+	++	++	0	++
39	Pregnancy.....	5	16	0	+	0	+	0	+
40	Male—ulcer of stomach.....	10	16	0	+	0	+	++	++
41	Pregnancy.....	10	16	0	+	+	+	+	+
42	Male—healthy.....	10	16	0	++	0	++	0	++
43	Pregnancy.....	10	20	0	+	++	++	++	++
44	Pregnancy.....	10	20	0	+	++	++	++	++
45	Male—healthy.....	10	20	0	+	0	+	+	+
46	Pregnancy.....	10	16	0	+	+	+	0	++
47	Pregnancy.....	10	16	0	+	0	+	0	+
48	Pregnancy.....	5	16	0	+	+	+	0	+
49	Pregnancy.....	10	18	0	++	+	+	+	++
50	Pregnancy.....	10	18	0	++	+	+	+	++
51	Pregnancy.....	10	18	0	+	0	+	++	++
52	Male—healthy.....	10	16	0	+	0	+	0	++
53	Male—healthy.....	10	16	0	+	0	+	0	++
54	Pregnancy.....	10	16	0	+	++	++	0	++
55	Pregnancy.....	10	16	0	0	++	++	0	++
56	Female — general cancer, not pregnant.....	10	20	0	++	0	++	+	++
57	Ca. uterus; not pregnant.....	10	20	0	+	+	+	++	++
58	Male—healthy.....	10	18	0	+	+	+	++	++
59	Pregnancy.....	10	18	0	++	++	++	++	++
60	Pregnancy.....	10	18	0	+	+	++	+	++
61	Male—Ca. ....	10	16	0	+	0	+	+	+
62	Male—Ca. ....	10	16	0	++	0	+	0	++
63	Male—Ca. ....	5	18	0	++	+	++	++	++
64	Male—Ca. ....	5	18	0	+	0	++	++	++
65	Male—Ca. ....	5	18	0	+	+	++	++	++
66	Male—Ca. ....	10	18	0	++	0	+	++	++
67	Male—Ca. ....	10	18	0	+	0	+	+	++

\* In addition to the symbols explained in the footnote to Table 1, in this and the following tables Ca. signifies carcinoma; (1), before incubation; (2), after incubation.



dried placenta (or dried carcinoma) were thoroughly mixed by vigorous shaking and toluene added to cover the mixture completely. The toluene was used to restrict bacterial growth and to prevent drying. These mixtures were incubated at 37.5 C. for twelve hours, and then one-half was drawn off, filtered and tested by the biuret reaction. The remainder was incubated for from four to twelve hours longer and again tested. The best results were obtained by the longer incubation (Table 1). Control tests were carried out with urine alone and with substrate in distilled water. The control urines were always positive to the ninhydrin reagent. All green and blue in the biuret test were considered negative. A positive test varied from purple to lilac.

TABLE 3.—PREGNANCY URINES INCUBATED WITH PLACENTAL AND CANCER SUBSTRATES

Specimen Number	C.c. Each Test	Hours Incubation	Control (1) Urine		Urine and Placenta		Urine and Carcinoma		Control (2) Urine	
			B.	N.	B.	N.	B.	N.	B.	N.
41	10	16	0	+	+	+	0	+	+	+
43	10	20	+	++	++	++	+	++	++	++
44	10	20	0	+	++	++	0	++	++	++
46	10	16	0	+	+	+	0	+	0	++
47	10	16	0	+	0	+	+	++	0	+
48	5	16	0	+	+	+	0	+	0	+
49	10	18	0	++	+	+	0	+	+	++
50	10	18	0	++	+	+	+	+	+	++
51	10	18	0	+	0	+	0	+	++	++
54	10	16	0	+	++	++	0	+	0	++

From Table 1 it is evident that the minimal period of incubation should not be less than twelve hours, which is considerably longer than noted by Kiutsi. He obtained his results in two hours. It was noteworthy that within twenty-four hours all the specimens except one became positive. The ninhydrin reaction became more positive as the period of incubation increased.

Forty-one pregnancy urines were incubated with placenta, as noted in Table 2, and only twenty-four, or 58.9 per cent., gave a positive reaction and seventeen, or 41 per cent., were negative. Of twenty-six specimens of urine from nonpregnant women examined with placenta, six, or 23 per cent., were positive and twenty, or 77 per cent., were negative. The negative results appeared to be more important than the positive findings.

The next step was to determine the specificity of the test by incubating pregnancy urines with placental and cancer substrates and then treating cancer urines the same way.

Ten pregnancy urines were incubated with cancer tissue and three gave a positive result, while seven gave a negative result. This again speaks for a greater value of negative results than of positive reports.

In malignancy with cancer substrate ten urines were used. These cases were positively carcinomatous, as shown by pathologic reports. In this study four were positive and six were negative. These were incubated at the same time with placental tissue and three, or 30 per cent., gave a positive reaction, while seven, or 70 per cent., gave a negative result.

TABLE 4.—CANCER URINES INCUBATED WITH PLACENTAL AND CANCER SUBSTRATES

Specimen No.	C.c. Each Test	Hours Incubation	Control (1) Urines		Urine and Placenta		Urine and Carcinoma		Control (2) Urine		Diagnosis
			B.	N.	B.	N.	B.	N.	B.	N.	
35	5	18	0	+	0	+	0	+	+	++	Ca. breast
56	10	20	0	++	0	++	+	+	+	++	General Ca.—woman, not pregnant
57	10	20	0	+	+	+	+	++	++	++	Ca. uterus, not pregnant
61	10	16	0	+	0	+	0	+	+	++	Ca. stomach—male
62	10	16	0	++	0	+	0	++	0	++	Ca. stomach—male
63	5	18	0	++	+	++	+	++	++	++	Ca. pancreas—male
64	5	18	0	+	0	++	+	+	++	++	Ca. stomach—male
65	5	18	0	+	+	++	0	+	++	++	Ca. stomach—male
66	10	18	0	++	0	+	0	+	++	++	Ca. breast
67	10	18	0	+	0	+	0	+	+	++	Ca. stomach—male

Of interest was the observation that pregnancy urines reacted with cancer tissue the same way that the cancer urines reacted with the placental substrate.

The biuret reaction was always negative before incubation in both the test and control urines. The reactions after incubation in the control urines gave the following conflicting results:

Total number control urines (nonpregnant patients) (including cancer urines).....	26
Positive biuret reactions after incubation.....	17
Negative biuret reactions after incubation.....	9
Total number pregnancy urine controls.....	41
Positive control reactions after incubation.....	23
Negative control reactions after incubation.....	18

The results so far obtained in these studies do not coincide with conclusions drawn by the originator of the test. We are inclined to believe that negative results alone should be used as corroborative diagnosis.

# THE RELATION OF PREGNANCY AND CHILDBIRTH TO PELLAGRA IN WOMEN \*

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NEW YORK

## INTRODUCTION

The exceptionally high prevalence of pellagra among women between the ages of 20 and 45 years has been pointed out in previous publications of this commission and is now generally recognized as characteristic of pellagra in the general population of the southern portion of the United States. In our First Progress Report<sup>1</sup> attention was directed to the observation that pregnancy seems to inhibit the development of pellagrous symptoms, and to the indication that childbirth<sup>2</sup> seems to predispose to an attack of the disease. In the present paper we wish to examine the correlation between the attack of pellagra, either initial or recurrent, on the one hand, and the existence of pregnancy and the event of childbirth on the other, in the histories of all the pellagrins in our series for whom there are definite records in regard to these phenomena.

## INITIAL ATTACK OF PELLAGRA DURING PREGNANCY

In the histories of 624 women of childbearing age who suffered their first attack of pellagra in a year prior to 1915 there are twenty definite records of onset of pellagra during pregnancy. For the 101 colored women counted in this group there are no such instances recorded. Excluding the 101 colored women from the present consideration, we find that for twenty, or 3.8 per cent., of the remaining

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\* From the Robert M. Thompson Pellagra Commission of the New York Post-Graduate Medical School and Hospital.

\* This paper has been written since Dr. Garrison and Dr. Siler were recalled to active duty in the Medical Corps, U. S. Navy, and the Medical Corps, U. S. Army, respectively. They are not personally responsible for the detailed compilation of data or for the specific deductions drawn from them.

1. Siler, J. F., and Garrison, P. E.: An Intensive Study of the Epidemiology of Pellagra, *Am. Jour. Med. Sc.*, 1913, **146**, 42, 238; First Progress Report of the Thompson-McFadden Pellagra Commission, 1914, p. 17.

2. Siler, J. F.; Garrison, P. E., and MacNeal, W. J.: Pellagra, a Summary of the First Progress Report of the Thompson-McFadden Pellagra Commission, *Jour. Am. Med. Assn.*, 1914, **62**, 8; First Progress Report, 1914, p. 3.



523 white women there is history of onset of pellagra during pregnancy. Brief notes for each of these cases follow.

Pellagrin 186, aged 22 at onset of pellagra in March, 1908, was in the second month of pregnancy when she suffered an initial attack of pellagra, manifested by scaling of the hands, stomatitis and diarrhea. The eruption subsided in about two weeks and the attack was evidently a mild one. During the following two years this patient remained entirely free from the disease, again becoming pregnant in August, 1909, and giving birth to a child in May, 1910. In April, 1911, and again in 1912 she suffered mild recurrences of pellagra, but remained free from symptoms of the disease in 1913 and 1914.

Pellagrin 277, aged 25 at the time of onset, first developed a pellagrous erythema in April, 1908, three months before the birth of her child. The attack was evidently mild, although complete, showing erythema of hands, sore mouth, dysentery and nervous symptoms. The eruption remained for about one week, and by winter there was a complete subsidence of all symptoms. In April, 1909, she again had a mild, though complete, attack. There was a very severe recurrence in March, 1910, during which the patient was bedridden for six weeks. In the following two years mild recurrences appeared in April and June, respectively. During 1913 and 1914 this patient remained entirely free from symptoms of pellagra.

Pellagrin 644, aged 26 at onset, was in the fourth month of pregnancy when pellagra first developed in April, 1909. The attack was mild, consisting of sore mouth, followed by an erythema, which subsided in two months. During the following three years she remained free from the disease, being pregnant through the entire pellagra season in 1911. In 1913 she suffered her first recurrence and it was a mild one. In 1914 she remained free from symptoms of pellagra.

Pellagrin 827, aged 33 at onset, was in the seventh month of pregnancy when pellagrous symptoms first developed in August, 1909. The attack was undoubtedly a severe one, for after the birth of her child in October, 1909, the patient was admitted to a hospital, where she died six weeks later.

Pellagrin 95, aged 26 at onset, was in the third or fourth month of pregnancy when she suffered an initial attack of pellagra about May, 1910. The attack was manifested merely by characteristic erythema on the hands. In May, 1911, the patient suffered a mild recurrence, with sore mouth and an erythema on the hands and arms, which subsided in about one week. During the winter of 1911 and also in 1912 she had some mental derangement, but had shown no further recurrence of erythema to July, 1912, the date of the last observation.

Pellagrin 18, aged 29 at the time of onset, developed an initial attack of pellagra in June, 1910, before the birth of her child, the exact date of delivery being unrecorded. The attack was mild, manifested by erythema on the forearms, sore mouth and dysentery, all of which symptoms subsided by July, 1910. In May, 1911, she suffered a severe recurrence with skin, gastro-intestinal and nervous symptoms, which persisted until the following September. Again in April, 1912, this patient had a complete recurrence of pellagra, which had no definite subsidence that year, but ended in death in January, 1913.

Pellagrin 62, aged 21 at onset, was in the fourth month of pregnancy when she first developed an erythema in July, 1911. The attack seems to have been rather severe, with sore mouth, an intermittent diarrhea and an eruption which lasted through most of the summer. In this case there was history of ill health following an operation for pelvic disease in 1909. Early in January, 1912, the patient gave birth to a child and in June, 1912, she suffered a mild recurrence, having been troubled all the spring with diarrhea and insomnia. In the summer of 1913 she had a pronounced attack while pregnant, giving birth to a child in October. In 1914 she is recorded as having recurrences of erythema

in February, May and August, suffering also from sore mouth, insomnia, constipation and loss of appetite during the attack in May.

Pellagrin 568, aged 23 at the time of onset, was in the ninth month of pregnancy when an initial erythema appeared in June, 1911. The patient suffered also from diarrhea, but within two months after the birth of the child the symptoms subsided. After the first attack in 1911 this patient remained quite free from the disease up to the date of our last observation, July, 1914.

Pellagrin 724, aged 24 at onset, suffered her first attack of pellagra in the fall of 1911, when in the first month of pregnancy. The attack consisted merely of the eruption on the hands, which subsided before winter. In June, 1912, after the birth of her child, the patient suffered a recurrence of erythema. She is without record for 1913, but is reported to have suffered a recurrence of erythema in the spring of 1914.

Pellagrin 280, aged 19 at the time of onset, became pregnant the first week in July, 1912, and suffered her initial attack of pellagra in that same month. The only symptom in this first attack was the erythema, which appeared on her hands and subsided by fall. In July, 1913, just three months after childbirth, she suffered a recurrence of pellagra, with erythema, stomatitis, diarrhea and sore tongue. In May, 1914, she had a recurrence of the skin symptoms and sore mouth.

Pellagrin 260, aged 22 at onset, was in the sixth month of pregnancy, when a pellagrous erythema first developed in April, 1912. Her child was born in August, 1912. In this case there were repeated exacerbations of the cutaneous eruption and persistent weakness throughout the fall and winter. She became worse in the summer of 1913. She was admitted to the hospital in Spartanburg and died there Sept. 7, 1913.

Pellagrin 531, aged 29 at the time of onset, was in the second month of pregnancy when she developed an initial attack of pellagra in August, 1912. At this time she had merely a slight erythema. After the birth of her child in March, 1913, she had a recurrence, beginning in May, manifested by erythema, sore tongue and dysentery. In 1914 she reported better health than she had had for years, and she remained free from symptoms during that year.

Pellagrin 701, aged 31 at onset, was in the eighth month of pregnancy when an erythema first appeared on her hands in March, 1912. The attack was a very slight one, evidenced merely by the pellagrous eruption. In the spring of 1913 she had a severe recurrence. In May, 1914, she suffered a recurrence of the skin eruption.

Pellagrin 253, aged 35 at onset, was in the eighth month of pregnancy when a pellagrous erythema first developed in May, 1912. The eruption, which was the only symptom in this initial attack, extended over the hands and forearms and subsided by August, 1912. The patient remained quite free from recurrence of the disease in 1913 and 1914.

Pellagrin 1167, aged 21 at onset, suffered an initial attack of pellagra in July, 1913, just one month before the birth of a child. The only symptom in this first attack was the typical erythema, which cleared up after her child was born. In May, 1914, she had a recurrence of the erythema.

Pellagrin 977, aged 22 at the time of onset, was in the eighth month of pregnancy when the initial erythema appeared in July, 1914. The symptoms consisted of eruption on the forearms and hands, with sore mouth and indigestion.

Pellagrin 1267, aged 28 at the time of onset, suffered her initial attack of pellagra a few days before the birth of her child in January, 1914. This attack was manifested by gastro-intestinal as well as skin symptoms, and these did not disappear until some time after the birth of the child.

Pellagrin 958, aged 29 at onset, had suffered indigestion for two months prior to the development of pellagra in February, 1914, three months before the birth of her child. In this case there was an early subsidence of skin symptoms, but diarrhea persisted to the date of last observation in July, 1914.

Pellagrin 1078, aged 42 at the time of onset, was well advanced in pregnancy



when pellagra first developed in the latter part of July, 1914. With the exception of the erythema, which recurred that summer, and sore mouth, the patient's health was not markedly impaired.

Pellagrin 964, age not recorded, suffered a first attack of pellagra in January, 1914, just before the birth of her child. The onset was mild, but in the following June the erythema recurred and with it stomatitis and marked general weakness.

TABLE 1.—SUMMARY OF THE TWENTY INITIAL ATTACKS OF PELLAGRA WHICH OCCURRED DURING PREGNANCY

Pellagrin Number	Age at Onset	Date of Onset	Month of Pregnancy	Character of Attack	History During Pregnancy
188	22	March, 1908	Second	Mild	Recovery
277	25	April, 1908	Sixth	Mild	Recovery
644	26	April, 1909	Fourth	Mild	Recovery
827	33	August, 1909	Seventh	Severe	Persisted*
95	26	May, 1910	Third or Fourth	Mild	Recovery
18	29	June, 1910	Middle	Mild	Recovery
62	21	July, 1911	Fourth	Severe	Recovery
568	23	June, 1911	Ninth	Mild	Recovery
724	24	Fall, 1911	First	Mild	Recovery
260	19	July, 1912	First	Mild	Recovery
260	22	April, 1912	Sixth	Severe	Persisted†
531	29	August, 1912	Second	Mild	Recovery
701	31	March, 1912	Eighth	Slight	Recovery
253	35	May, 1912	Eighth	Mild	Recovery
1167	21	July, 1913	Eighth	Mild	Recovery
977	22	July, 1914	Eighth	Mild	Recovery
1267	28	January, 1914	Ninth	Mild	Recovery
958	29	February, 1914	Sixth	Mild	Recovery
1078	42	July, 1914	"Advanced"	Mild	Recovery
964	Not recorded	January, 1914	Ninth	Mild	Recovery

\* This patient died of pellagra six weeks after childbirth in the fall of 1909.

† This patient survived the year, giving birth to her child in August, 1912. She did not regain her health, but continued to suffer from pellagra until death in September, 1913.

Among the 523 recorded initial attacks of pellagra in white women in the age period 12 to 49 years there are only twenty, or 3.8 per cent., in which the onset occurred during pregnancy. None of these twenty died of pellagra during pregnancy. In fact, with a single exception, those who developed the disease early in pregnancy suffered only mild attacks. This exceptional case, Pellagrin 62, had been in ill health following a pelvic operation in 1909 and she suffered a rather severe initial attack of pellagra in July, 1911, in the fourth month of pregnancy. In one instance, Pellagrin 827, the attack appeared in the seventh month of pregnancy, persisted until childbirth and ended in



death six weeks later. In another case, Pellagrin 260, pellagra appeared in the sixth month of pregnancy and persisted until long after delivery, the patient dying of pellagra in the following year. The comparative infrequency<sup>3</sup> of onset of pellagra during pregnancy and the benign character of the attack when it does occur indicate that during pregnancy there is an increased resistance to pellagra.

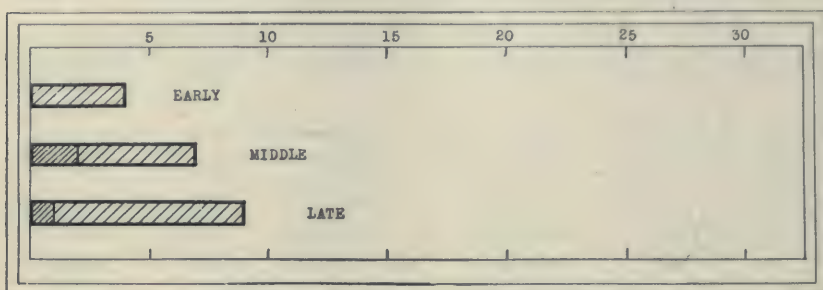


Fig. 1.—Initial attacks of pellagra which began during the first, second and last three months of pregnancy, respectively. The total length of the bar indicates the number of instances of onset in the respective three-month period. The more thickly shaded portion indicates the number of instances in which the attack of pellagra was severe.

#### THE RELATION OF CHILDBIRTH TO INITIAL ATTACK

Frequently in the histories of pellagrous women childbirth just previous to the initial attack is recorded as a possible predisposing factor. On closer examination of the records of these mothers the rather large number of instances in which a childbirth in the early part of the year, usually from January to April, has been followed within a few months, or even weeks, by an initial development of pellagrous symptoms invites consideration. In the records of the 624 pellagrous women of childbearing age (12 to 49 years) in our series to the end of 1914 there are fifty-seven who gave a history of childbirth within six months preceding onset. This group of 624 women includes 101 colored pellagrins. Because of the comparatively small number of records for the colored race and doubtless, also, because of the incompleteness of the records, there are recorded but four cases of possible predisposing childbirth, or 4 per cent., for the colored race. After subtracting from the total group of women the 101 colored pellagrins,

3. The condition of pregnancy exists at any given time in more than 3.8 per cent. of the women between 12 and 49 years of age in this population. If only 3.8 per cent. of them were pregnant at a given time on the average, then the average woman would be pregnant 3.8 per cent. of 38 years (12 to 49, inclusive), or 1.44 years, about seventeen months, and would, therefore, fail to bring forth more than two children during her childbearing period, a number too small to maintain the population and unquestionably far below the actual average number in this population.

we find that fifty-three, or 10.1 per cent., of the remaining 523 white women suffered onset of pellagra following a childbirth.

If the total birth records in Spartanburg County for a given year were available for comparison with the total number of initial attacks of pellagra among mothers delivered in the respective year, it would be highly interesting to ascertain in what ratio women who had recently borne a child were attacked by this disease. We have, however, the histories of the 523 white women who did suffer attack. The fact that a very large proportion of all women in these southern mill villages do bear children and that standards of living and environmental influences are generally similar reduces them to a fairly uniform basis for comparison.

TABLE 2.—COMPARISON OF THE NUMBER OF WOMEN FROM 12 TO 49 YEARS OF AGE, WITH ONSET OF PELLAGRA IN EACH YEAR, WITH THE NUMBER HAVING RECORD OF CHILDBIRTH JUST PRIOR TO ONSET OF THE DISEASE

Year of Onset	White Women			Colored Women		
	Total Initial Attacks	Instances of Childbirth Just Prior to Onset		Total Initial Attacks	Instances of Childbirth Just Prior to Onset	
		Number	Per Cent.		Number	Per Cent.
Before 1908.....	28	3	10.7	4	0	0.0
1908.....	13	1	7.5	1	0	0.0
1909.....	30	1	3.4	2	0	0.0
1910.....	70	7	10.0	12	0	0.0
1911.....	117	9	7.7	14	0	0.0
1912.....	80	5	6.3	20	0	0.0
1913.....	114	20	17.5	23	1	4.3
1914.....	71	7	9.9	25	3	12.0
Total.....	523	53	10.1	101	4	4.0

In Table 2 are summarized the total number of incident cases of pellagra among women in the age period 12 to 49 years at the time of onset of pellagra, together with the total recorded cases of childbirth prior to onset among these women. These are grouped according to race and year of onset. That 10.1 per cent. of the pellagrous white women suffered onset of pellagra following childbirth suggests very strikingly that the puerperal state may predispose to pellagra.<sup>4</sup>

4. Less than 10 per cent. of the women in this population, on the average, will have borne a child within six months. If 10 per cent. of them should have borne a child within six months, at any given time, it would require that the average woman bring forth one child every five years, or a little more than seven children in the thirty-eight years (12 to 49, inclusive) of her assumed childbearing period. This figure is unquestionably too large for the average woman of this population.

This hypothesis may be strengthened by a detailed examination of the histories of these mothers. In the citations which follow there have been recorded, so far as the records permit, any complicating disorders which might have influence on the physical condition of the mother prior to onset of pellagra. The cases are grouped for consideration according to the year of onset, with the exception of those incident in a year prior to 1908. The latter are considered in one group. Of these, twenty-eight were of childbearing age at time of onset and for three of them there is history of childbirth within a period of six months preceding the onset of pellagra.

Pellagrin 354 was 16 years old when she suffered her first attack of pellagra in the summer of 1901, following childbirth. In this case is recorded the complication of puerperal insanity, which appeared just prior to development of pellagra. The initial attack was a severe one, in which skin, gastro-intestinal and mental symptoms were well marked. The patient gave a history of recurrent erythema and mental disturbance nearly every summer to death in February, 1910.

Pellagrin 1024, aged 23 at onset, had her initial attack in the summer of 1905, following a childbirth. For this case there is a history of freedom from attack for four years subsequent. But in 1910 she again developed frank symptoms and continued to suffer recurrences each summer to 1914, inclusive.

Pellagrin 543, aged 36 at onset, suffered her first attack of pellagra in the spring of 1906, about two months after childbirth. She suffered annual recurrence to 1913. In 1914 she remained free from recurrence up to the time of her last observation, which was made in July.

Of the thirteen white women of childbearing age with onset of pellagra in 1908, there is one for whom there is recorded a childbirth preceding the onset of pellagra.

Pellagrin 1024, aged 23 at onset, had her initial attack in the summer of 1905, 1908. In July of the same year symptoms of pellagra first developed and persisted to her death in January, 1909.

In 1909 there were thirty instances of initial attack of pellagra among white women of childbearing age. For one of these there is a history of childbirth prior to onset.

Pellagrin 59, aged 35 at onset, first developed symptoms of pellagra in March, 1909, three months after the birth of her child. At this time she suffered from loss of appetite, insomnia and nervousness. The erythema in this first attack did not appear until June. She suffered a recurrence each summer thereafter to 1914, during which year she remained free from symptoms.

Of the seventy white women of childbearing age who suffered initial attacks in 1910, there were seven who gave a history of childbirth prior to attack of pellagra.

Pellagrin 702, aged 23 at onset, suffered her first attack in October, 1910. After the birth of twins earlier in the year menstruation had not reappeared. She suffered recurrences in 1911 and 1912. In 1913, after a childbirth in April, she had a recurrence of symptoms in May. In April, 1914, she also suffered recurrence.



Pellagrin 544, aged 23 at the time of onset, first developed pellagra in the fall of 1910, about one month after the birth of her child. In 1911 she gave birth to a child in the summer and escaped recurrence of symptoms during that year and also during 1912; but a few days after childbirth in April, 1913, she suffered a recurrence. There is no record for 1914.

Pellagrin 112, aged 24 at onset, gave birth to a child in March, 1910. The following June she suffered her first attack of pellagra. In 1911 she had a recurrence, but escaped in 1912. In April, 1913, while she was pregnant, she suffered a recurrence. In 1914 she remained free from symptoms.

Pellagrin 998, aged 27 at the time of onset, had her first attack of pellagra in June, 1910, just one month after the birth of a child. This patient gave a history of indigestion for several months before the birth of her child and of sore mouth, persistent diarrhea and insomnia prior to the development of the erythema. Her ill health continued till death in June, 1911.

Pellagrin 860, aged 32 at onset, first developed an erythema in August, 1910, shortly after giving birth to a child. During 1911, 1912 and 1913 she remained quite free from symptoms of pellagra. In 1914, however, while she was again pregnant, she suffered a recurrence in February, about five weeks before her child was born. The pellagrous symptoms persisted and the patient died in June, 1914.

Pellagrin 292, aged 39 at onset, gave birth to a child in January, 1910. She had a definite attack of pellagra in the following June. She suffered recurrences each year to 1914, during which year she is said to have remained free from symptoms.

Among the 117 white women of childbearing age incident in 1911, there are nine instances of onset following a childbirth.

Pellagrin 269, aged 23 at onset, suffered her initial attack in May, 1911, just three weeks after the birth of her child. She had recurrences each year thereafter to 1914, inclusive. In 1913, while she was pregnant, pellagrous symptoms developed in May, two months before the birth of her child.

Pellagrin 980, aged 25 at onset, gave birth to a child in June, 1911. At that time she was suffering from severe stomatitis and the erythema appeared soon after. The mental symptoms became marked in this case, and after she had made an attempt to take the life of her child, a nurse was in constant attendance. The pellagrous symptoms recurred in 1912 and persisted until death in August of the same year.

Pellagrin 678, aged 26 at onset, gave birth to a child in March, 1911. In the following summer symptoms of pellagra first developed. In 1912 she was pregnant during the pellagra season, giving birth to a child in October. She remained quite free from symptoms during that year and also during 1913 and 1914.

Pellagrin 122, aged 27 at time of onset, suffered an initial attack of pellagra two or three weeks after the birth of her child in March, 1911. She had no recurrence of symptoms to 1914, inclusive. In 1912 she was pregnant through the greater part of the pellagra season, giving birth to a child in August.

Pellagrin 81, aged 27 at onset, gave birth to a child in May, 1911. She suffered from diarrhea for a month before the baby was born, and the erythema appeared in June. In 1912 she had a recurrence of erythema. She remained quite free from symptoms during 1913 and 1914.

Pellagrin 883, aged 30 at the time of onset, first developed an erythema five weeks after the birth of her child in March, 1911. She remained free from symptoms during 1912, but suffered a recurrence in 1913. In 1914 she escaped recurrence.

Pellagrin 281, aged 32 at onset, gave birth to a child in February, 1911. Her baby had persistent bowel trouble and died at the age of 4 months. In July, 1911, shortly after the death of the child, the mother had her initial attack of pellagra. She remained free from symptoms of the disease in 1912, during which

year she was pregnant until November. She also escaped recurrence in 1913, but suffered an attack in 1914.

Pellagrin 1137, aged 34 at onset of pellagra in the spring of 1911, gives a history of the appearance of pellagrous symptoms following the birth of her two children in 1911 and 1913, respectively. In 1912 and 1914 she remained free from the disease.

Pellagrin 238, aged 38 at onset, gave birth to a child in February, 1911, and suffered her first attack of pellagra in the following May or June. After that she remained entirely free from symptoms up to the last observation in June, 1914.

Of the eighty white women of childbearing age with onset in 1912, there are five with definite record of childbirth just prior to the initial attack of pellagra.

Pellagrin 596, aged 19 at onset, gave birth to a child in December, 1911. The following spring (1912) she had her initial attack of pellagra. In July, 1913, she had a recurrence. She was pregnant in 1914 from February and remained free from symptoms during that year.

Pellagrin 614, aged 27 at time of onset, suffered her initial attack of pellagra shortly after giving birth to a child in August, 1912. In 1913 and 1914 she suffered complete, though mild, recurrences in May and March, respectively. In 1914 she was pregnant until July.

Pellagrin 1000, aged 29 at onset, was in poor health after the birth of her last child in January, 1912. The following March (1912) the erythema appeared for the first time. In March, 1913, she suffered a recurrence of symptoms and she died in April, 1913.

Pellagrin 164, aged 30 at the time of onset, suffered an initial attack of pellagra in June, 1912, two months after the birth of a child. In 1913 she was pregnant from June. She remained free from symptoms during 1913. In February, 1914, she gave birth to a child, but up to June of that year had suffered no recurrence.

Pellagrin 1185, aged 35 at onset, gave birth to a child in January, 1912. She had her initial attack in the following April (1912). In 1913 she remained free from symptoms, but suffered a recurrence in 1914.

In 1913 there were 114 white women of childbearing age who had an initial attack of pellagra. For twenty-one of these there is a record of a childbirth prior to onset.

Pellagrin 798, aged 18 at time of incidence in June, 1913, had given birth to a child three months before. She died of pellagra in July, 1913.

Pellagrin 635, aged 20 at onset, had given birth to a child late in December, 1912. Pellagra developed the following April (1913). During 1914 she remained free from symptoms.

Pellagrin 872, aged 22 at the time of onset, gave birth to a child in May, 1913. The following June she developed an initial erythema. In 1914 she remained free from symptoms.

Pellagrin 549 was 23 years old when she had her first attack of pellagra in June, 1913. She had given birth to a child the preceding March. She remained free from recurrence the following year.

Pellagrin 1034, aged 23 at onset, suffered a first attack of pellagra in August, 1913, just one month after giving birth to a child. She had a recurrence in July, 1914.

Pellagrin 837, aged 23 at the time of onset, gave birth to a child in March, 1913. She developed pellagrous symptoms for the first time in August, 1913. In 1914 she escaped recurrence.



Pellagrini 741, aged 24 at onset, suffered her initial attack of pellagra in June, 1913, two months after a childbirth. She had a severe recurrence in May, 1914.

Pellagrini 639, aged 24 at onset, gave birth to a child in February, 1913. The following May (1913) she had her first attack of pellagra. In 1914 she was reported to have had a severe recurrence during the spring and summer.

Pellagrini 552, aged 24 at onset, gave birth to a child in January, 1913. The following May (1913) she developed pellagra for the first time. In 1914 she remained free from symptoms.

Pellagrini 559, aged 24 at onset, gave birth to a child in February, 1913. She suffered her initial attack the following May. In 1914 she escaped recurrence.

Pellagrini 904, aged 25 at onset, gave birth to a child in September, 1913. Her health was very poor after childbirth, and in December, 1913, she developed symptoms of pellagra. She suffered a recurrence of the disease in 1914.

Pellagrini 576, aged 25 at onset, gave birth to a child in December, 1912. She had poor health during the months following, and in June, 1913, suffered her first attack of pellagra. In 1914 she had a slight recurrence of symptoms.

Pellagrini 591, aged 26 at onset, was nursing her 5-months-old baby when pellagra first developed in June, 1913. She is without record for 1914.

Pellagrini 458, aged 26 at onset, gave birth to a child in January, 1913. She began to feel ill in March, but continued to nurse her baby until pellagra developed in May, 1913. She remained free from symptoms during 1914.

Pellagrini 498, aged 28 at onset, suffered her first attack in June, 1913, about three months after the birth of her child. During 1914 she was pregnant until September. She remained free from symptoms of pellagra in 1914.

Pellagrini 700, aged 29 at onset, gave birth to a child in May, 1913, and pellagra developed at about the same time. She grew progressively worse and was admitted to the hospital on August 10, where she died two weeks later.

Pellagrini 1031, aged 29 at onset, had suffered from stomatitis in the fall of 1912. She gave birth to a child in January, 1913, and diarrhea developed shortly afterward, followed by a severe erythema in July. These symptoms persisted until death in October, 1913.

Pellagrini 537, aged 29 at onset, was in poor health before and following the birth of her child in October, 1912. In March, 1913, she developed an erythema for the first time. During 1914 she was pregnant until September, and remained free from symptoms during that year.

Pellagrini 665, aged 30 at onset, had an initial attack in May, 1913. Following the birth of her child three months before, she had been in poor health. During 1914 she remained free from symptoms.

Pellagrini 610, aged 32 at the time of onset, gave birth to a child in December, 1912. In the following June (1913) she suffered her initial attack of pellagra. In 1914 she escaped recurrence of symptoms.

Pellagrini 294, aged 36 at onset, gave birth to a child in February, 1913, and had her first attack of pellagra in April. She grew worse and was admitted to the Good Samaritan Hospital in July. Against the advice of her physician she left the hospital in August and died in October, 1913.

In 1914 there were seventy-one incident cases of pellagra recorded among white women of childbearing age. For seven of the white women, or 9.9 per cent., there is record of a possibly predisposing childbirth.

Pellagrini 1250, aged 20, had a severe attack of dysentery in May, 1914, about two weeks after the birth of her child. The pellagrous erythema did not appear until the second week in October.

Pellagrini 922, aged 22 at onset, gave birth to a child the last of February, 1914. She developed initial symptoms of pellagra the last of April.



Pellagrin 1155, aged 22 at time of onset, gave birth to a child in the latter part of January, 1914. She first developed erythema in the following July.

Pellagrin 954, aged 26 at onset, suffered an initial attack in June, 1914, about six weeks after a childbirth.

Pellagrin 1019, aged 27, gave birth to a child early in May, 1914, and first developed pellagrous symptoms about two weeks after delivery.

Pellagrin 1011, aged 29 at onset, gave birth to a child the last of May, 1914, and two months later suffered an initial attack of pellagra. In the case of this patient there was a complication of diarrhea which had persisted from the fall of 1913.

Pellagrin 1161, aged 33 at time of onset, gave birth to a child in March, 1914, and developed initial pellagrous symptoms about four weeks after delivery.

TABLE 3.—PELLAGRINS WHO SUFFERED THE INITIAL ATTACK WITHIN ONE MONTH AFTER CHILDBIRTH

Pellagrin Number	Age at Onset	Date of Childbirth	Onset of Pellagra	Interval Indicated by the Original Record
<b>White Women—</b>				
854.....	16	Summer, 1901	Summer, 1901	Following childbirth
1024.....	23	Summer, 1905	Summer, 1905	Following childbirth
1107.....	18	Summer, 1908	July, 1908	Following soon after
544.....	23	Fall, 1910	Fall, 1910	About one month after childbirth
998.....	27	May, 1910	June, 1910	One month
860.....	32	August, 1910	August, 1910	Following childbirth
269.....	23	May, 1911	May, 1911	Three weeks after childbirth
980.....	25	June 5, 1911	June, 1911	Soon after childbirth
122.....	27	March, 1911	Late March, 1911	Two or three weeks
81.....	27	May 8, 1911	June, 1911	About one month
1137.....	34	Spring, 1911	Spring, 1911	Following childbirth
614.....	27	August, 1912	September, 1912	Following childbirth
1034.....	23	July 1, 1913	August, 1913	One month
700.....	29	May, 1913	May, 1913	At about same time
1019.....	27	May, 1914	May, 1914	About two weeks
1161.....	33	March, 1914	April, 1914	Four weeks

Of the 101 colored women incident before 1915, there is a history of a previous childbirth for four.

Pellagrin 410, aged 25 at onset, first developed symptoms of pellagra in the spring of 1913, following a childbirth, the exact date of which is not recorded. She died of pellagra in July, 1913.

Pellagrin 943, aged 21, gave birth to a child in January, 1914. During the early months of her pregnancy she had lived with her grandmother, Pellagrin 539, who died of pellagra during the summer of 1913. The patient remained free from symptoms during that year. In May, 1914, however, four months after the birth of her child, she developed pellagra for the first time.

Pellagrin 1094, aged 21 at onset, gave birth to a child early in June, 1914. In August she suffered an initial attack of pellagra.

Pellagrin 923, aged 24 at the time of onset, developed pellagra for the first time in May, 1914, about four or five months subsequent to a childbirth. The symptoms persisted until death, June 24, 1914.

Of the ninety-six women aged 12 to 49 years with initial attack of pellagra in 1914, there are, therefore, ten instances of a possibly predisposing childbirth, or 10.4 per cent., for both races. If these incident cases with their proportion of prior childbirths are added to those of women incident before 1914 with their respective cases of childbirth, we have a total of fifty-seven possibly predisposing childbirths among 624 pellagrous women, or a percentage of 9.1 for both races. This

TABLE 4.—PELLAGRINS WHO SUFFERED THE INITIAL ATTACK LATER THAN ONE MONTH BUT WITHIN THREE MONTHS AFTER CHILDBIRTH

Pellagrin Number	Age at Onset	Date of Childbirth	Onset of Pellagra	Interval Indicated by the Original Record
<b>White Women—</b>				
543.....	36	Early in 1906	Spring, 1906	About two months
702.....	23	Late summer or fall, 1910	October, 1910	About three months
112.....	24	March 19, 1910	June, 1910	Three months
883.....	30	March 21, 1911	April, 1911	Five weeks
1000.....	29	January, 1912	March, 1912	Two months
164.....	30	April, 1912	June, 1912	Two months
1185.....	35	January, 1912	April, 1912	Three months
798.....	18	March, 1913	June, 1913	Three months
872.....	22	May 10, 1913	July, 1913	Two months
549.....	23	March, 1913	June, 1913	Three months
741.....	24	April, 1913	June, 1913	Two months
639.....	24	February, 1913	May, 1913	Three months
559.....	24	February, 1913	May, 1913	Three months
904.....	25	September, 1913	December, 1913	Three months
498.....	28	March, 1913	June, 1913	Three months
665.....	30	February, 1913	May, 1913	Three months
294.....	36	February, 1913	April, 1913	Two months
922.....	22	February, 1914	April, 1914	Two months
954.....	26	May, 1914	June, 1914	Six weeks
1011.....	29	May, 1914	July, 1914	Two months
<b>Colored Women—</b>				
410.....	25	Early in 1913	Spring, 1913	Not recorded
1094.....	21	June, 1914	August, 1914	Two months

indicated percentage is undoubtedly too small, for the records of very many of these 624 women are incomplete, especially in respect to dates of childbirths. Indeed it was not until the year 1913 that the subject of the possible predisposition of puerperal women to pellagra was given any particular attention. Thus, we find the tabulations for that year and for 1914 more complete and, therefore, more reliable criteria in regard to this relationship than are the recorded data for the pre-

ceding years. In this connection it should be mentioned that the records for the latter part of 1914 are incomplete because of the termination of the field work of the commission in the fall of that year.

The fifty-seven examples of onset of pellagra within six months after childbirth are presented in Tables 3, 4 and 5, separately, according to the length of the interval between childbirth and the onset of the initial attack. Those with onset of pellagra in the first month

TABLE 5.—PELLAGRINS WHO SUFFERED THE INITIAL ATTACK LATER THAN THREE MONTHS BUT WITHIN SIX MONTHS AFTER CHILDBIRTH

Pellagrin Number	Age at Onset	Date of Childbirth	Onset of Pellagra	Interval Indicated by the Original Record
<b>White Women—</b>				
59.....	35	December, 1908	June, 1909	Six months
292.....	39	January, 1910	June, 1910	Five months
678.....	26	March, 1911	Summer, 1911	Not recorded
281.....	32	February, 1911	July, 1911	Five months
238.....	38	February, 1911	May or June, 1911	Three or four months
596.....	19	December, 1911	Spring, 1912	Not recorded
635.....	20	Dec. 22, 1912	April, 1913	Four months
837.....	23	March, 1913	August, 1913	Five months
552.....	24	January, 1913	May, 1913	Four months
576.....	25	December, 1912	June, 1913	Six months
591.....	26	January, 1913	June, 1913	Five months
458.....	26	Jan. 10, 1913	May, 1913	Four months
1031.....	29	January, 1913	July, 1913	Six months
537.....	29	October, 1912	March, 1913	Five months
610.....	32	December, 1912	June, 1913	Six months
1250.....	20	May, 1914	October, 1914	Five months
1155.....	22	January, 1914	July, 1914	Six months
<b>Colored Women—</b>				
943.....	21	January, 1914	May, 1914	Four months
923.....	24	January or February, 1914	May, 1914	Three or four months

after delivery are shown in Table 3. These number sixteen, all white women, and constitute 32.2 per cent. of the white women in the whole group. In Table 4 are shown the twenty-two pellagrins who suffered initial attack in the second and third months following childbirth. Twenty of these were white women, making up 37.7 per cent. of the fifty-three white women in the whole group. In Table 5 are shown nineteen pellagrins who developed the disease in the fourth, fifth or sixth month following childbirth, seventeen of these being white women. This group is approximately equal to that shown in Table 3,



indicating that onset of pellagra has been about three times as common in the first month following pregnancy as in either the fourth, fifth or sixth month following such an event. Furthermore, this group shown in Table 5 is one half as large as the sum of those in Tables 3 and 4, indicating that onset of pellagra has been about twice as frequent in the first three months following childbirth as it has been in the succeeding three months. These relationships certainly suggest that the event of childbirth may be an important factor in predisposing to pellagra and that it may also determine to some degree the onset of definite symptoms of the disease.

Another interesting relation shown by these tables is that of season. Of the sixteen childbirths recorded in Table 3, no less than fifteen occurred in the spring or summer months. On the other hand, of the nineteen childbirths shown in Table 5, only two occurred during spring

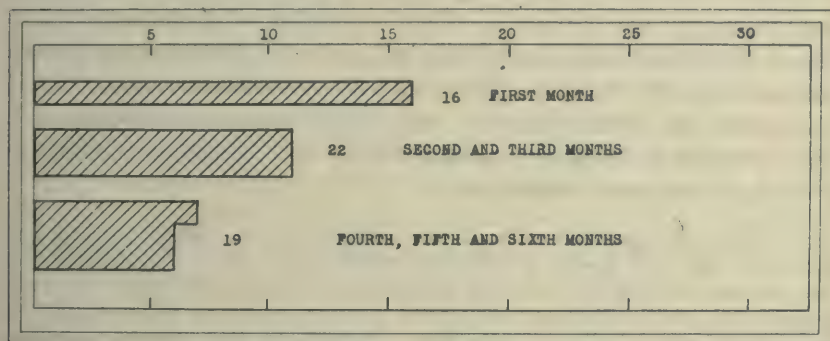


Fig. 2.—The number of initial attacks of pellagra following childbirth. Note that there were sixteen in the first month, twenty-two in the second and third months and nineteen in the fourth, fifth and sixth months after delivery.

or summer and no less than fourteen of them took place in the three winter months, December, January and February. It is evident that the danger of pellagra developing within a few weeks after childbirth is greatest when the pregnancy terminates during the spring or summer and, from what has been said above, it would seem that the cases of this kind are the ones to which childbirth has the most definite relation.

#### THE RELATION OF RECURRENT ATTACK OF PELLAGRA TO PREGNANCY AND CHILDBIRTH

In the preceding discussion the relation of the first or initial attack of pellagra to certain physiologic conditions of the woman have been considered. In this section we purpose to consider the subsequent behavior of all women who survived the year of initial attack and who presented a definite record of subsequent pregnancy or childbirth,

in order to ascertain whether there is any correlation, either positive or negative, between the pregnant state and recurrence of pellagra or between the puerperal state and recurrence of pellagra. In the preceding paper<sup>5</sup> of this series has been presented the correlation between recurrence of pellagra, on the one hand, and race, sex and age, on the other, and it was there suggested that pregnancy and childbirth seemed to influence the behavior of the women in respect to recurrence. In that paper all the women who suffered the initial attack of pellagra previous to the year 1914 were considered. Here, those of this same group for whom there is a definite record of pregnancy or of childbirth after the onset of pellagra will be considered.

There are, in all, eighty-three white women and four colored women in this category, including fifteen<sup>6</sup> of the twenty women who suffered the initial attack of pellagra during pregnancy. For a considerable part of these, however, there is a definite record of more than one pregnancy per patient, so that the number of instances of pregnancy or of childbirth in surviving pellagrins available for the present discussion is 101 in white women and five in colored women.

Among the white pellagrins with onset previous to 1908 there is only one with a definitely recorded history of pregnancy or childbirth subsequent to onset of pellagra.

Pellagrin 70, aged 27, suffered her initial attack in 1905. Every spring thereafter she had bowel trouble, sore mouth and nervous disturbance, but the evidence of definite recurrent erythema is lacking until 1913. She was pregnant in 1907 or 1908 and miscarried, but the record of this pregnancy is not definite enough to be of value here. In 1913 she gave birth to a child in April, suffered a definite recurrence of pellagra in August and died in January, 1914, of pellagra and complicating disorders.

Of the white pellagrins who survived the initial attack in 1908 there are five with definite record of pregnancy or of childbirth after the onset of pellagra.

For the history of Pellagrin 186 see the section on Initial Attack During Pregnancy.

Pellagrin 432, aged 24, suffered her first attack of pellagra in 1908, with severe recurrence in 1909, but no recurrence in 1910. She became pregnant in August, 1910, giving birth to her child in May, 1911. The years 1911 and 1912 were passed without reappearance of pellagra. She again conceived in August, 1912, and was delivered in May, 1913. This time an attack of pellagra

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5. Siler, J. F., Garrison, P. E., and MacNeal, W. J.: The Relation of Recurrent Attacks of Pellagra to Race, Sex and Age of the Patient and to Treatment of the Disease, *THE ARCHIVES INT. MED.*, 1916, **18**, 652.

6. For three cases with onset of pellagra in 1914 during pregnancy, namely, Pellagrins 977, 958 and 1078, data subsequent to delivery are not available. For two cases, Pellagrins 827 and 260, with initial attack during pregnancy, pellagrous symptoms persisted after delivery. These five cases are excluded from this category.

appeared during the puerperium and there was another definite recurrence in 1914.

For history of Pellagrin 277 see the section on Initial Attack During Pregnancy.

Pellagrin 987 was 25 years old in the summer of 1908, when her pellagrous symptoms first appeared. In September, 1908, she became pregnant. She suffered a mild recurrence of pellagra the following spring before childbirth, which occurred June 10, 1909. Every spring thereafter to 1914, inclusive, she had recurrences, mild symptoms developing in 1912 during a pregnancy, which was terminated by childbirth in November, 1912.

Pellagrin 37, aged 32 at the time of onset, suffered her first attack in the fall of 1908. Each spring thereafter she had a recurrence, although in 1911, after an early recurrence, she remained entirely free from symptoms after April, when she became pregnant, being delivered the following December. In April, 1912, while still nursing her baby, she suffered another recurrence, and pellagra again recurred the following spring (1913). In August, 1913, she again became pregnant and remained free from symptoms of pellagra in 1914 until after childbirth on May 15. At our last observation in June, 1914, slight but definite signs of a recurrence of pellagra were already present.

Of the seven instances of pregnancy subsequent to onset of pellagra there are, therefore, five in which the pregnancy terminated in the early summer and two in which childbirth occurred in the late fall. Of the five pregnancies terminating in the summer, four were free from recurrence of pellagrous symptoms, while in one instance a mild attack of pellagra developed in the sixth or seventh month. In the two instances of childbirth in the fall the patient remained quite free from the disease during pregnancy in one and in the other a mild recurrence appeared in the first or second month of pregnancy.

There were two instances of a childbirth following onset during pregnancy. In one of these cases the childbirth occurred in July, in the other it occurred in the fall. There were, therefore, nine instances of childbirth subsequent to onset of pellagra, six occurring in the summer and three in the fall. In seven of these the mother remained entirely free from symptoms for a period of at least three months following her delivery, and in two there was recurrence in the summer within four weeks after the childbirth.

Of the white pellagrins with initial attack in 1909, there are nine for whom definite records of pregnancy, or of childbirth subsequent to onset, are available.

Pellagrin 817, aged 17 at onset in 1909, became pregnant soon after and remained entirely free from any symptoms of pellagra up to our last observation in 1914.

Pellagrin 94 suffered her first attack in April, 1909, when 21 years of age. The following year she had a recurrence in April. In 1911 she suffered a very severe recurrence, being confined to bed for three months. In July, 1911, she became pregnant, giving birth to a child in March, 1912. In this case a pregnancy during the cool season was succeeded by a very mild recurrence in June, 1912. Late in the summer of 1912 she became pregnant and aborted in October, 1912. In February, 1913, she became pregnant again and



remained entirely free from symptoms for that year. In 1914 she again suffered a recurrence of pellagra.

Pellagrin 178 was 23 years old at the time of her initial attack in the spring of 1909. She recovered and remained free from symptoms until 1912. After childbirth early in 1912 she suffered a recurrence of pellagra in March and in 1913 she had another mild recurrence. In January, 1914, she became pregnant and escaped recurrence of pellagra to the date of the last observation, July 7, 1914.

Pellagrin 151 suffered her first attack in March, 1909, when 23 years of age. Each subsequent spring she had a recurrence up to and including 1912. In July, 1912, she became pregnant. Her baby was born in March, 1913. She remained entirely free from symptoms of pellagra during 1913 and 1914.

Pellagrin 119, aged 25, had her initial attack in July, 1909. The following March she became pregnant and remained free from symptoms of pellagra during 1910. In 1911 she had no recurrence and again became pregnant in August. After a winter pregnancy, terminated by childbirth April 29, 1912, she remained quite free from symptoms during 1912, but in 1913 she suffered a recurrence. She is without record in 1914.

Pellagrin 140, aged 25, suffered her first attack in May, 1909. She had a recurrence in 1910, and in 1911 the symptoms reappeared in April. In June, 1911, she became pregnant. After giving birth to a child in February, 1912, she had a very severe recurrence in June, 1912, and died of pellagra on Aug. 17, 1912.

Pellagrin 80 was 26 years old when she suffered her initial attack early in the summer of 1909. In 1910 she had no recurrence of pellagra, but in July, 1911, while she was pregnant, she had a very severe recurrence and after delivery in August, 1912, her hands broke out a second time. Thereafter, from 1912 to 1914, inclusive, she was entirely free from symptoms of the disease.

For history of Pellagrin 644 see section on Initial Attack During Pregnancy.

Pellagrin 1346, aged 30 at the time of onset of pellagra in 1909, suffered recurrences in 1910 and 1911. In 1912 she was pregnant until September and during that year remained free from symptoms. In 1913 she also escaped recurrence of the erythema, although she had sore mouth and some physical weakness. She is without record for 1914.

Among the white pellagrins with initial attack in 1909 there were, therefore, thirteen instances of pregnancy subsequent to onset of pellagra. In five cases childbirth occurred in the early months of the year (from January to April, inclusive), in one case it occurred in late summer and in six instances the period of pregnancy extended over the entire pellagra season, with termination in the fall. In one instance the exact season of gestation was not recorded. With one exception the patients were entirely free from symptoms of pellagra during all these pregnancies. In the case of the pregnancy with summer termination, however, a severe recurrence was suffered in the eighth month and after childbirth in August a second erythema developed within four weeks.

There was one case of initial attack during pregnancy with subsequent childbirth, making a total of fourteen instances in this category in 1909. For one 1914 pregnancy records of subsequent history are not available. For ten of the thirteen remaining cases there was no recurrence of pellagra for three months subsequent to delivery; two

of the instances of childbirth early in the year were followed by recurrence within three months and the one instance of childbirth late in the summer was followed by a recurrence of pellagra within one month.

Among the white pellagrins with initial attack in 1910 there are seventeen with a definite history of pregnancy or of childbirth subsequent to onset.

Pellagrin 824, aged 17 at onset of pellagra in 1910, suffered a mild recurrence in 1911, apparently during a pregnancy which began in April. Her child was born in December. In 1912, 1913 and 1914 she remained free from recurrence of pellagra.

Pellagrin 750 was 18 years of age when she had her initial attack in 1910. For 1911 and 1912 there are no definite records of recurrence. In July, 1912, she became pregnant and gave birth to a child in March, 1913. The following August she suffered a mild recurrence and again had a renewal of symptoms in 1914.

Pellagrin 275 had her first attack of pellagra in the spring of 1910, when she was 18 years old. She has had a recurrence each year thereafter up to and including 1914. In 1913 she became pregnant in March, suffered a mild recurrence of pellagra in June and was delivered the following December. This is an instance of recurrence of pellagra coincident with pregnancy during the summer season.

Pellagrin 1153, aged 19 at the time of onset of pellagra in the summer of 1910, remained entirely free from symptoms of pellagra during the four years subsequent. In 1913 a childbirth was recorded, but the season of pregnancy was not noted.

Pellagrin 702, aged 23, suffered her first attack in October, 1910. She had recurrences each year thereafter. She gave birth to a child in April, 1913, and suffered a recurrence of pellagra in May, 1913.

Pellagrin 544, aged 23, suffered her first attack in the fall of 1910, about one month after childbirth. In 1911 she was free from symptoms. She also gave birth to a child in the summer of that year, but the exact month of delivery is not recorded. In 1912 she was also free from recurrence of pellagra, but in 1913 after giving birth to a child in April she promptly developed pellagrous symptoms. For 1914 the record is uncertain.

Pellagrin 112, aged 24, first developed symptoms of pellagra in June, 1910, a few weeks after childbirth. In 1911 she suffered a recurrence and also had an induced abortion at six months in December. She remained free from symptoms during 1912, again becoming pregnant in December, 1912. In April, 1913, she suffered a mild recurrence while still pregnant. The child was born in August of the same year. In 1914 the patient remained free from symptoms.

Pellagrin 218, aged 25, had her initial attack in the summer of 1910, the exact month of onset being unrecorded. In June, 1910, she became pregnant, giving birth to a child in February, 1911. Early in 1911 she had a recurrence of pellagra, but the exact date is not recorded. In April of the same year she again became pregnant and the child was born in January, 1912. The following March she suffered her second recurrence. In May, 1913, pellagrous symptoms again developed. In July, 1913, she became pregnant a third time, and five weeks after delivery, on March 28, 1914, she suffered her fourth recurrence of pellagra. Here, then, are three instances of recurrence following within three months after childbirth.

Pellagrin 282, aged 26, had her initial attack in September, 1910. She had a recurrence in September, 1911, but in 1912 she remained free from symptoms.



In 1913, after a childbirth in July, she had a recurrence of pellagra with two eruptions of the erythema, and she also had a renewal of symptoms in 1914.

For history of Pellagrin 95 see section on Initial Attack During Pregnancy.

Pellagrin 127, aged 28 at onset of pellagra in 1910, suffered a recurrence the following year, but remained free from symptoms in 1912. In 1913 she became pregnant in May and escaped recurrence that year. In 1914, after a childbirth in February, she suffered a recurrence of symptoms in the early summer.

Pellagrin 171 was 28 years old when she had her initial attack of pellagra in October, 1910. She had a recurrence in April, 1911, but in 1912 she was pregnant from January and the symptoms of pellagra did not appear. She was again pregnant from January, 1913, and remained free from the disease during that year. In 1914, however, she had a mild recurrence.

Pellagrin 279 was 29 years of age at onset of pellagra in July, 1910. She had a recurrence in 1911, but remained free from symptoms during 1912 and 1913. In February, 1914, she became pregnant and escaped recurrence in that year to the date of the last observation, July 20, 1914.

For history of Pellagrin 18 see section on Initial Attack During Pregnancy.

Pellagrin 860, aged 32, developed her first erythema in August, 1910, shortly after childbirth. During the three following years she remained free from symptoms. In February, 1914, she suffered a severe recurrence about five weeks before the birth of her child and she died of pellagra two months after delivery.

Pellagrin 6, aged 32, had her initial attack in April, 1910. The following October she became pregnant and remained free from symptoms in 1911, being delivered in June. In 1912 she had a recurrence and died Aug. 21, 1912.

Pellagrin 29, aged 40 at the time of onset in 1910, suffered a recurrence in 1911 and 1912. In 1913 she remained free from symptoms and became pregnant in the fall of the same year. She gave birth to a child in May, 1914, and escaped recurrence during that year also.

There are, therefore, among the white pellagrins incident in 1910 nineteen instances of pregnancy subsequent to onset of pellagra. Seven of these terminated early in the year, five in the summer, six in the fall months and for one the exact season was not recorded. Of the pregnancies with termination in the spring, five were quite free from manifestations of pellagra, a severe recurrence in the eighth month was suffered in one and for the seventh there was no definite history. Of the five pregnancies with childbirth in the summer, four were free from symptoms and a mild recurrence in the fifth month of gestation occurred in the fifth case. Of the six instances of pregnancy with fall termination, three were entirely free from symptoms, mild recurrences in the early months were suffered in two and for the sixth there was no definite history.

Among the white pellagrins incident in 1910 there were two cases of onset during pregnancy and one case in which the onset month was uncertain, with subsequent childbirth. For these three cases of childbirth subsequent to onset, as well as for eighteen of the nineteen instances of pregnancy just mentioned above, the pellagrous history of the mother following delivery may be considered. For one 1914 pregnancy subsequent history is not available, because our field observations ceased in the fall of that year. Of the twenty-one instances



of childbirth subsequent to initial attack of pellagra in 1910, therefore, eight occurred in the spring, five in the summer, seven in the fall and for one the exact season was not recorded. In six instances of delivery in the spring a recurrence of pellagra appeared within three months after childbirth, in one case the mother remained free from symptoms and in one instance a severe recurrence during late pregnancy persisted till death two months after delivery. Of the five instances of childbirth in the summer, all but one were free from recurrence of symptoms during the following three months. Of the seven instances of childbirth in the fall and winter, none was followed by recurrence of pellagra within three months.

There are twenty-two white pellagrins with initial attack in 1911 who have a record of pregnancy or of childbirth subsequent to onset of pellagra.

Pellagrin 896 was 19 years old when she had her first attack in April, 1911. In the following September she became pregnant and gave birth to a daughter in May, 1912. Near the termination of this pregnancy, in April, 1912, she suffered a mild recurrence of pellagra. She had recurrence also in 1913 and 1914.

For history of Pellagrin 62 see section on Initial Attack During Pregnancy.

Pellagrin 269, aged 23, suffered her first attack of pellagra in May, 1911, just three weeks after the birth of her child. She had a recurrence in May, 1912. That same year she became pregnant in November and the following spring she suffered a mild recurrence before her child was born. She also had a recurrence in 1914.

For history of Pellagrin 568 see section on Initial Attack During Pregnancy.

For history of Pellagrin 724 see section on Initial Attack During Pregnancy.

Pellagrin 726 was 25 years old when she suffered her first attack of pellagra in May, 1911. In January, 1912, she became pregnant and she remained free from pellagrous symptoms during that year. She also escaped recurrence in 1913, but in June, 1914, while she was again pregnant, she suffered a mild recurrence of symptoms three weeks before the birth of her child.

Pellagrin 678, aged 26 at onset, suffered her initial attack in the summer of 1911, following a childbirth in March. In February, 1912, she again became pregnant, giving birth to a son in October. She remained free from symptoms of pellagra during that entire year and she remained free from recurrence of the disease up to the last observation in May, 1914.

Pellagrin 1033, aged 27 at onset, had her initial attack in the spring of 1911. The year following she suffered a recurrence in March. In September, 1912, she became pregnant and the following spring mild pellagrous symptoms again appeared before the birth of her child in May, 1913. She also had a recurrence in 1914.

Pellagrin 122, aged 27, suffered her initial attack the latter part of March, 1911, in which month she had given birth to a child. In December, 1911, she again became pregnant and escaped recurrence of the disease during 1912. She also remained free from symptoms during 1913 and 1914.

Pellagrin 21 was 27 years of age when she suffered her first attack in May, 1911. The following July she became pregnant and after a childbirth in March, 1912, she had a recurrence of pellagra in May. In 1913 she also had a recurrence in March, which subsided by July. This patient died March 4, 1914, without recurrence.

Pellagrin 879 had her initial attack in April, 1911, when she was 29 years of age. In March, 1912, she gave birth to a daughter. She escaped recurrence

during 1912. She also remained free from symptoms of pellagra in 1913, in which year she again became pregnant in April. She suffered a recurrence of pellagra in April, 1914.

Pellagrin 281 was 32 years of age at the time of onset of pellagra in July, 1911. In February, 1912, she became pregnant and escaped recurrence for that year. She also remained free from symptoms in 1913, but suffered a recurrence in 1914.

Pellagrin 84, aged 32 at the time of her initial attack in 1911, became pregnant in October of that same year. She suffered a recurrence in July, 1912, only a few weeks after the birth of her child. In 1913 and 1914 she was free from symptoms.

Pellagrin 822 was 33 years of age when she suffered her first attack in 1911. She escaped recurrence in 1912, in which year she became pregnant in July. After a childbirth in March, 1913, she also escaped recurrence for that year. In June, 1914, the pellagrous symptoms again appeared.

Pellagrin 274 suffered her first attack in June, 1911, when she was 33 years of age. She became pregnant early in 1912 and remained quite free from symptoms during that year. In 1913 she also escaped recurrence, but in 1914 she had a recurrence of pellagra and she died with complications in September, 1914.

Pellagrin 1137 was 34 years of age when she had her initial attack in the spring of 1911. This patient gives a history of the appearance of pellagrous symptoms after childbirth in 1911 and 1913. The years during which she did not bear children, namely, 1912 and 1914, she remained free from recurrence of the pellagrous symptoms.

Pellagrin 1038, aged 34 at onset of the disease in the summer of 1911, remained free from recurrence during the two years following, although her general health was poor in 1912. In 1913 she gave birth to a child in September, and she was not well thereafter. She suffered a recurrence of pellagra in July, 1914.

Pellagrin 599 had her initial attack in 1911, when 36 years of age. She remained free from symptoms the following year, but suffered a recurrence in July, 1913. In January 1914, she became pregnant and remained free from symptoms during that year up to our last observation in June.

Pellagrin 565, aged 36 at onset in the spring of 1911, suffered a recurrence in April of the following year. In May, 1913, while she was pregnant, she had a mild recurrence of pellagra. The record of this case for 1914 is incomplete.

Pellagrin 604 had her initial attack in the spring of 1911, when 37 years of age. She escaped recurrence in 1912 and also in 1913, in which year she gave birth to a daughter in May. In 1914 she suffered a mild recurrence of pellagra in June.

Pellagrin 212, aged 40 at onset in May, 1911, was pregnant throughout the spring and summer months of 1912. She escaped recurrence during that year and remained entirely free from symptoms in 1913 and 1914.

Pellagrin 278 was 40 years of age when she suffered her initial attack in July, 1911. She had recurrences in 1912 and 1913. In January, 1914, she became pregnant and remained free from symptoms up to our last observation, July 20, 1914.

Of the twenty-two instances of pregnancy subsequent to onset of pellagra in 1911, three terminated in the early part of the year, seven in the summer, eleven in the fall and for one the season was not recorded. In all three of the instances of childbirth in the early part of the year the patient remained free from symptoms of pellagra during the period of pregnancy; in four of the seven instances of termination in the summer mild recurrences were suffered late in pregnancy; of the eleven instances of childbirth in the fall, there were



two with summer recurrence during pregnancy, one mild and one pronounced, while nine were free from symptoms.

Among the white pellagrins incident in 1911 there were three who were pregnant at the time of onset of pellagra. For these three instances of childbirth subsequent to onset, as well as for the twenty-two instances of pregnancy subsequent to onset cited above, the history of the mother in the three months following delivery may be considered. Of these twenty-five instances of childbirth, four occurred in the early months of the year and for only one of these was there history of recurrence of pellagra within three months. Of the nine instances of childbirth in the summer, only three were followed by recurrence within three months. Of the eleven instances of a delivery in the fall months, nine were recorded as free from symptoms in the months immediately following, while for two 1914 cases the records were incomplete because our field observations ceased in the fall of that year.

There were sixteen white pellagrins who suffered their initial attack in 1912 and whose records of childbirth or of pregnancy in subsequent years are sufficiently definite to be utilized in this discussion.

Pellagrin 593 was 16 years old at the time of onset in August, 1912. She was pregnant throughout the spring and summer months of 1913, giving birth to a child in August, 1913. She escaped recurrence in 1913, but suffered a mild attack of pellagra in 1914.

Pellagrin 55 had her initial attack in June, 1912, when 19 years of age. She remained free from symptoms of pellagra in 1913, during which year she was pregnant from April. Her history for 1914 is incomplete.

For history of Pellagrin 280 see section on Initial Attack During Pregnancy.

Pellagrin 596 gave birth to a child in December, 1911. The following spring (1912) when she was 19 years old, her first attack of pellagra developed. She suffered a recurrence of the disease in 1913. In February, 1914, she became pregnant again and remained free from symptoms of pellagra for the year 1914 to our last observation in June.

Pellagrin 884 suffered her initial attack of the disease in the summer of 1912, when she was 20 years of age. The following summer she had a severe recurrence of pellagra. She became pregnant in June, 1913. The childbirth in March, 1914, was followed by a severe recurrence of symptoms in May.

Pellagrin 562, aged 23 at onset, suffered her initial attack of the disease in the spring of 1912, the exact month of onset being unrecorded. In April, 1912, she became pregnant, giving birth to a child the following December. She had a recurrence of pellagrous symptoms in May, 1913. In 1914, however, she was pregnant until well into July and remained free from symptoms up to the time of our last observation in the early part of July, 1914.

Pellagrin 102 was 24 years of age when she had her initial attack in May, 1912. She remained quite free from symptoms in 1913. In March, 1914, she became pregnant and had also escaped recurrence in that year to the date of our last observation, in October, 1914.

Pellagrin 833, aged 26 at onset in April, 1912, became pregnant in November of the same year. In May, 1913, while she was still pregnant, a mild recurrence of pellagra developed. She suffered recurrence also in 1914.

Pellagrin 614, aged 27 at onset, suffered her first attack in September, 1912,



shortly after the birth of a child. In 1913 and 1914 she had complete but mild recurrences. In 1914 the symptoms appeared in March, when she was five months pregnant.

For history of Pellagrin 531 see section on Initial Attack During Pregnancy.

Pellagrin 164, aged 30, gave birth to a child in April, 1912. Pellagrous symptoms developed for the first time in the following June. She became pregnant again in June, 1913, giving birth to a child in February, 1914. She remained free from recurrence during 1913 and 1914.

For history of Pellagrin 701 see section on Initial Attack During Pregnancy.

Pellagrin 439 suffered her initial attack in April, 1912, at the age of 32 years. She had a recurrence of symptoms in April, 1913. Through the spring and early summer of 1914 she was pregnant and escaped recurrence during that year to our last observation, May 25, 1914.

Pellagrin 721, aged 32 at time of onset, suffered her initial attack in 1912, the exact month being unrecorded. She became pregnant in July, 1912, and gave birth to a child in April, 1913. In September of the same year she suffered a recurrence, but remained free from symptoms up to the time of our last observation in May, 1914.

For history of Pellagrin 253 see section on Initial Attack During Pregnancy.

Pellagrin 813, aged 35 at time of onset in 1912, became pregnant in February, 1913, and suffered a mild recurrence the following summer. For 1914 the record of this case is uncertain.

There were, therefore, eleven instances of pregnancy subsequent to onset of pellagra among white women with initial attack in 1912. Of these, two terminated in the early part of the year, in one instance of which there was no recurrence of symptoms, while the history in the other was indefinite. There were five instances of pregnancy with termination in the summer, in three of which the patient remained quite free from symptoms, while in the other two, mild recurrences of pellagra appeared during pregnancy. Of the four instances of termination in the fall, the patient remained free from symptoms in three, while in the fourth instance a mild recurrence appeared during pregnancy in the summer.

Among the white pellagrins incident in 1912 there were four with initial attack during a pregnancy and two with subsequent childbirth, but for whom the onset month was indefinite. These six cases of childbirth following onset may be added to the eleven instances of pregnancy subsequent to onset, giving seventeen instances of childbirth subsequent to onset of pellagra. Of these, five occurred in 1914 too late for subsequent observation in our field work, which terminated in the fall of that year. Of the remaining twelve instances of childbirth, six occurred in the early part of the year, a recurrence following within three months in three, while in the other three cases the mother remained quite free from symptoms. In three instances the childbirth occurred in the summer and in each case the mother escaped recurrence of symptoms in the three months immediately following. The three remaining instances of delivery occurred in the fall, the mother remaining free from recurrence for three months subsequent in each case.

Of the white pellagrins who suffered an initial attack in 1913, there are eleven with definite record of pregnancy or of a childbirth subsequent to onset.

Pellagrin 613 was 21 years old when she suffered her initial attack of pellagra in 1913. She was pregnant during the spring and early summer of 1914, being delivered prematurely in July. In this case the delivery at the seventh month of pregnancy did not seem to lessen her apparent resistance to attack during that year, for she remained quite free from symptoms of pellagra up to our last observation, July 20, 1914.

For history of Pellagrin 1167 see section on Initial Attack During Pregnancy.

Pellagrin 600, aged 26 at onset in July, 1913, became pregnant late in the fall. In 1914 she remained free from recurrence during the period of pregnancy. After giving birth to twins in July, she suffered a mild recurrence of pellagra in August.

Pellagrin 873 was 27 years of age when she had her initial attack in 1913, the exact month of onset being unrecorded. In the spring of the same year she became pregnant, giving birth to a child in January, 1914. In April, 1914, she suffered a recurrence of symptoms.

Pellagrin 498, aged 28 at onset, gave birth to a child about three months before the pellagrous symptoms first appeared in 1913. In January, 1914, she again became pregnant and remained quite free from recurrence for that year to the date of the last observation, Sept. 12, 1914.

Pellagrin 997 had her first attack in July, 1913, when 29 years of age. She became pregnant in February, 1914, and remained free from symptoms for that year to the date of the last observation, July 20, 1914.

Pellagrin 537, aged 29 at the time of onset in March, 1913, had been in poor health following her last confinement in October, 1912. In January, 1914, she again became pregnant and escaped recurrence of pellagrous symptoms during 1914 to the date of our last observation, Sept. 2, 1914.

Pellagrin 450 suffered her initial attack in the spring of 1913, when 30 years of age. In 1914 she was pregnant and remained quite free from symptoms up to the last observation in June.

Pellagrin 1207, aged 31 at the time of onset in 1913, became pregnant in the latter part of that year and gave birth to a child in June, 1914. She, however, did not escape pellagrous symptoms in 1914, and suffered a mild recurrence in the spring, while still pregnant.

Pellagrin 889 suffered her first attack in May, 1913, when 31 years of age. One week after the birth of a daughter in April, 1914, she suffered a recurrence of pellagra.

Pellagrin 518, aged 39 at the time of onset in February, 1913, remained free from symptoms in 1914. She was pregnant during the greater part of the pellagra season, giving birth to a child in June or July, 1914.

For histories of Pellagrins 1267 and 964, with onset in 1914, see section on Initial Attack During Pregnancy.

Among the white pellagrins with initial attack in 1913 there were, therefore, nine instances of subsequent pregnancy, of which one terminated in the spring, five in the summer and three in the fall. In the case of the pregnancy with termination in the spring the patient remained entirely free from symptoms of pellagra during the period of gestation. Of the five pregnancies with childbirth in the summer, there was entire freedom from symptoms in four, while in the fifth a mild recurrence of pellagra appeared in the seventh month. For the



three pregnancies with termination in the fall there was apparently complete freedom from symptoms of pellagra during pregnancy.

There was one instance of onset during pregnancy in 1914 and one of uncertain onset date with subsequent childbirth. Adding these two cases of childbirth subsequent to onset to the foregoing nine instances of pregnancy following onset, we have eleven instances of childbirth subsequent to initial attack of pellagra for consideration. Six of these instances of childbirth occurred in 1914, too late for subsequent observations. Of the remaining five, two occurred in the early months of the year, with recurrence of pellagra following in each case; and the other three took place in the summer, a recurrence following in one case, while in the other two the mother escaped recurrence in the three months immediately following.

Among the white pellagrins with initial attack previous to 1915, there were, therefore, eighty-two instances of pregnancy subsequent to onset and eighty-five instances of childbirth after onset of pellagra.

Of the colored pellagrins who suffered the initial attack previous to 1915, there are only four with definite history of pregnancy or childbirth subsequent to onset.

Pellagrin 109 had her first attack in June, 1911, when she was 29 years old. A childbirth in April, 1912, was followed by puerperal fever, and in May the pellagrous symptoms again appeared. A second childbirth in the summer of 1913 was likewise followed by a recurrence of pellagra, of which the patient died in the fall of 1913.

Pellagrin 67, aged 15 at the time of onset in May, 1912, gave birth to an illegitimate child in May, 1913, and suffered a severe recurrence of pellagra, which appeared before accouchement and persisted for several weeks afterward. In 1914 she had another severe recurrence of pellagra of which she died in June, 1914.

Pellagrin 183 was 17 years old at the time of onset of pellagra in June, 1912. She was pregnant during the spring and summer of 1913 and gave birth to a child on Sept. 18, 1913. During 1913 she remained free from recurrence of the disease. She is without record for 1914.

Pellagrin 733, aged 27 at onset of pellagra in 1913, gave birth to a child on May 20, 1914, and appears, from her record, to have remained free from recurrence during that year.

There are in this small group five instances of pregnancy subsequent to onset among colored pellagrins, one terminating in the spring, three in the summer and one in the fall. In four instances the patient remained quite free from symptoms during the period of gestation; in the fifth case a severe recurrence was suffered late in pregnancy and the symptoms persisted after the birth of the child in May. For four of these five instances of pregnancy there is history during the months subsequent to delivery. In one 1914 case further data are not available. Recurrence within three months appeared after the two childbirths which occurred in the spring and summer, respectively. In the case of one summer childbirth, symptoms which had appeared



during pregnancy persisted for several weeks afterward. For the instance of delivery in the fall there was no record of recurrence within three subsequent months.

In the records of all these pellagrins there were eighty-seven instances of pregnancy beginning subsequent to onset of pellagra and there were eighty-eight instances of childbirth taking place subsequent to the initial attack of the disease. The instances in these two categories are not equal in number, because the first contains sixteen<sup>7</sup> instances of pregnancy during 1914 for which there are no records for the months following childbirth, and which, therefore, cannot be utilized in the second category. Moreover, the second group includes not only fifteen of the twenty cases of onset during pregnancy for which there is history subsequent to delivery, but also four cases in which it is uncertain whether the initial attack occurred before or during pregnancy, but for which subsequent history of childbirth and puerperium is available. Furthermore, two instances of pregnancy with recurrence can be utilized in the first category, but must be omitted from the second, since the recurrent pellagrous symptoms persisted for many weeks after delivery in each case.

#### RELATION OF PREGNANCY TO RECURRENT PELLAGRA

Those patients who survived the initial attack of pellagra present definite records of eighty-seven instances of pregnancy subsequent to the onset of pellagra. Eighty-two of these pregnancies occurred in white women. In three of these the record in regard to recurrence of pellagra is uncertain. In seventeen of the remaining seventy-nine instances, or 21.5 per cent., a recurrent attack of pellagra appeared during the pregnancy, while in sixty-two instances, or 78.5 per cent., the disease did not recur during pregnancy. The colored race presents five instances of pregnancy, a recurrence of pellagra appearing during pregnancy in only one instance, 20.0 per cent. of the five. Table 6 shows a summary of these observations. It is at once evident that the pregnant pellagrins have been very much less liable to recurrence than pellagrous women in general, for whom, as was shown in the preceding paper of this series, the annual recurrence rate was 63 per cent.

The data in regard to the eighteen recurrences of pellagra during pregnancy are summarized in Table 7. In these eighteen recurrences there was not one instance of death from pellagra during the period of pregnancy. In one case, however, the mother died of pellagra shortly after the birth of her child. In three other cases the attack was

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7. For ten of these instances of pregnancy in 1914 no recurrence was recorded to the date of the last observation late in the respective period. We feel justified, therefore, in utilizing these in the pregnancy group.

TABLE 6.—BEHAVIOR DURING PREGNANCY OF PELLAGRINS WHO HAD PREVIOUSLY SUFFERED THE INITIAL ATTACK

Race	Record Uncertain	Record Definite	Recurrent Attacks		Escapes from Recurrence		Total
			Number	Per Cent.	Number	Per Cent.	
White.....	3	79*	17	21.5	62	78.5	82*
Colored.....	0	5	1	20.0	4	80.0	5
Total.....	3	84*	18	21.4	66	78.6	87*

\* This number includes ten pregnancies in 1914 in which no recurrences were noted to the date of the last observation.

TABLE 7.—SUMMARY OF THE EIGHTEEN RECURRENT ATTACKS OF PELLAGRA WHICH OCCURRED DURING PREGNANCY

Pellagrin Number	Age at Recurrence	Date of Recurrence	Month of Pregnancy	Character of Attack	History During Pregnancy
987	26	Spring, 1909	Late	Mild	Recovery
987	29	Spring, 1912	Early	Mild	Recovery
80	28	July, 1911	Eighth	Mild	Recovery*
824	18	1911	Early	Mild	Recovery
275	21	June, 1913	Fourth	Mild	Recovery
112	27	April, 1913	Sixth	Mild	Recovery
860	36	February, 1914	Eighth	Severe	Persisted†
896	20	April, 1912	Eighth	Mild	Recovery
62	23	Summer, 1913	Middle	Severe	Recovery
269	25	May, 1913	Seventh	Slight	Recovery
726	28	June, 1914	Ninth	Mild	Recovery
1033	29	April, 1913	Eighth	Mild	Recovery
565	38	May, 1913	Seventh	Mild	Recovery
833	27	May, 1913	Seventh	Mild	Recovery
614	29	March, 1914	Fifth	Mild	Recovery
813	36	Summer, 1913	Late	Slight	Recovery
1207	32	April, 1914	Seventh	Mild	Recovery
67	16	Spring, 1913	Late	Severe	Persisted‡

\* Some weeks after childbirth in August, the erythema reappeared on the hands.

† The attack of pellagra persisted after the birth of her child and the patient died of pellagra in June, 1914.

‡ The attack of pellagra appeared before childbirth and persisted for a considerable time afterward, with recovery. The patient died in a subsequent recurrence of pellagra in June, 1914.

undoubtedly severe, although followed by complete recovery for that year. One of these three patients, Pellagrin 80, gave a history of only the one recurrence after her initial attack in 1909. In the case of another, Pellagrin 62, the disease recurred regularly each year after onset in 1911; while in the case of the third, Pellagrin 67, a colored woman, the second recurrence in the year following the childbirth ended in death. There remain fourteen instances, or 77.8 per cent. of the total number of recurrences during pregnancy, in which the attack of pellagra was mild.

TABLE 8.—DISTRIBUTION OF RECURRENCES OF PELLAGRA DURING PREGNANCY ACCORDING TO MONTH OF GESTATION

	Definite Month									Month Uncertain		
	1	2	3	4	5	6	7	8	9	Early	Middle	Late
Period of gestation.....	1	2	3	4	5	6	7	8	9	Early	Middle	Late
Number of recurrences.....	0	0	0	1	1	1	4	4	1	2	1	3

The distribution of the recurrent attacks according to the month of gestation is shown in Table 8. The bulk of the attacks of pellagra appeared after the sixth month. Furthermore, the three severe attacks observed are recorded as occurring in the middle of the period of

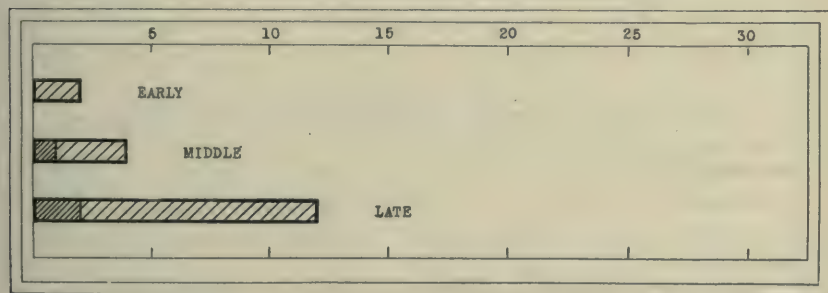


Fig. 3.—Recurrent attacks of pellagra during the first, second and last three months of pregnancy. The total length of the bar indicates the number of instances of recurrence beginning in the respective three-month period. The more thickly shaded portion indicates the number of instances in which the attack of pellagra was severe.

gestation, late in pregnancy and in the eighth month of pregnancy, respectively. It would appear, therefore, that the protective influence of pregnancy is greater in the earlier months.

In Table 9 the eighty-seven instances of pregnancy in pellagrins are summarized according to the season of the year in which the pregnancy terminated or was expected to terminate. From this tabulation it is evident that the season of the year plays a part in determining whether recurrence of pellagra shall take place during pregnancy.



TABLE 9.—BEHAVIOR OF PELLAGROUS WOMEN IN RESPECT TO RECURRENCE OF PELLAGRA DURING PREGNANCY OF PELLAGRINS WHOSE PREGNANCIES TERMINATED IN DIFFERENT SEASONS

Behavior during Pregnancy	White Women		Colored Women		Both Races	
	Number	Per Cent.	Number	Per Cent.	Number	Per Cent.
With pregnancy terminating from January to April, inclusive						
Recurrence.....	1	6.3	0	0.0	1	5.9
No recurrence.....	15	93.7	1	100.0	16	94.1
Total definite.....	16	100.0	1	100.0	17	100.0
Uncertain.....	2	.....	0	.....	2	.....
Total.....	18	.....	1	.....	19	.....
With pregnancy terminating from May to August, inclusive						
Recurrence.....	11	37.9	1	33.3	12	37.5
No recurrence.....	18	62.1	2	66.7	20	62.5
Total definite.....	29	100.0	3	100.0	32	100.0
Uncertain.....	0	.....	0	.....	0	.....
Total.....	29	.....	3	.....	32	.....
With pregnancy terminating from September to December, inclusive						
Recurrence.....	5	16.1	0	0.0	5	15.6
No recurrence.....	26	83.9	1	100.0	27	84.4
Total definite.....	31	100.0	1	100.0	32	100.0
Uncertain.....	1	.....	0	.....	1	.....
Total.....	32	.....	1	.....	33	.....
With time of termination of pregnancy uncertain						
Recurrence.....	0	0.0	0	.....	0	0.0
No recurrence.....	3	100.0	0	.....	3	100.0
Total definite.....	3	100.0	0	.....	3	100.0
Uncertain.....	0	.....	0	.....	0	.....
Total.....	3	.....	0	.....	3	.....

When the pregnancy began in the early summer, namely, from April to July, inclusive, and terminated in the period from January to April, inclusive, recurrence of pellagra rarely appeared during the course of the pregnancy. This period, of course, corresponds with the nine months of the year in which onset of pellagra is least frequent in the general population. The pregnancies which began in the period August to November, inclusive, or, roughly, the autumn months, and terminated in the period from May to August, inclusive, showed the greatest frequency of recurrent attacks of pellagra, namely, 37.5 per cent. In these cases the later months of pregnancy coincided with those months of the year in which the attack of pellagra is most usually initiated in the general population, namely, April, May and June. The pregnancies of the third group began in the period from December to March, inclusive, or, roughly, the winter months, and terminated in

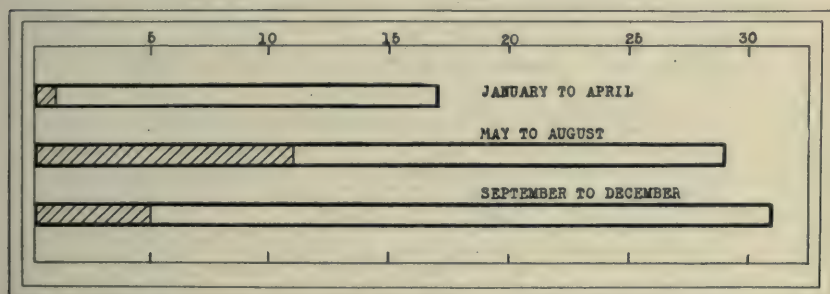


Fig. 4.—Recurrence of pellagra during pregnancy ending in different seasons. The number of instances of childbirth in each four-month period is indicated by the total length of the bar. The number of instances in each group in which recurrence of pellagra appeared during the period of gestation is indicated by the shaded portion of the respective bar.

the period September to December, inclusive. All of these pregnancies extended throughout the pellagra season. Nevertheless, in only 15.6 per cent. of them did recurrence of the disease appear during pregnancy. The months of greatest pellagra incidence, namely, April, May and June, here coincided with the earlier months of pregnancy rather than the later months, so that the low recurrence rate here supports the suggestion that the protective influence of pregnancy is more pronounced in the earlier rather than the later part of the period of gestation.

#### RELATION OF CHILDBIRTH TO RECURRENT PELLAGRA

There are eighty-eight instances of childbirth among pellagrins for whom there are records subsequent to the childbirth. The behavior during the three months following childbirth in these eighty-eight instances is summarized in Table 10. The frequency of recurrence in

TABLE 10.—BEHAVIOR OF PELLAGRINS DURING THE THREE MONTHS FOLLOWING CHILDBIRTH

Race	Recurrent Attacks		Escapes from Recurrences		Total
	Number	Per Cent.	Number	Per Cent.	
White.....	21	24.7	64	75.3	85
Colored.....	2	66.7	1	33.3	3
Total.....	23	26.1	65	73.9	88

TABLE 11.—BEHAVIOR OF PELLAGROUS WOMEN IN RESPECT TO RECURRENCE OF PELLAGRA DURING THE THREE MONTHS FOLLOWING CHILDBIRTH IN DIFFERENT SEASONS

Behavior after Childbirth	White Women		Colored Women		Both Races	
	Number	Per Cent.	Number	Per Cent.	Number	Per Cent.

## With childbirth occurring from January to April, inclusive

Recurrence.....	13	50.0	1	100.0	14	51.9
No recurrence.....	13	50.0	0	0.0	13	48.1
Total.....	26	100.0	1	100.0	27	100.0

## With childbirth occurring from May to August, inclusive

Recurrence.....	7	25.0	1	100.0	8	27.6
No recurrence.....	21	75.0	0	0.0	21	72.4
Total.....	28	100.0	1	100.0	29	100.0

## With childbirth occurring from September to December, inclusive

Recurrence.....	0	0.0	0	0.0	0	0.0
No recurrence.....	28	100.0	1	100.0	29	100.0
Total.....	28	100.0	1	100.0	29	100.0

## With season of childbirth uncertain

Recurrence.....	1	33.3	0	.....	1	33.3
No recurrence.....	2	66.7	0	.....	2	66.7
Total.....	3	100.0	0	.....	3	100.0



the first three months after childbirth, namely 26.1 per cent., is enormously high for a period of three months, just one quarter of a year. It may be compared with the annual recurrence rate of 63 per cent. for the total female pellagrins of Spartanburg County after dividing this latter figure by four, which yields a recurrence rate of 15.8 per cent. for a period of three months. It may also be compared with the recurrence rate during the nine months of pregnancy shown in the preceding section of this paper, namely, 21.4 per cent., after this latter figure has been divided by three, giving 7.1 per cent. as the recurrence rate for a period of three months. In comparison with either of these, the recurrence rate in the three months following childbirth, namely, 26.1 per cent., stands out conspicuously.

The behavior of these pellagrous women during the three months following childbirth is shown in Table 11, according to the season of the year in which the childbirth occurred. It is at once evident that

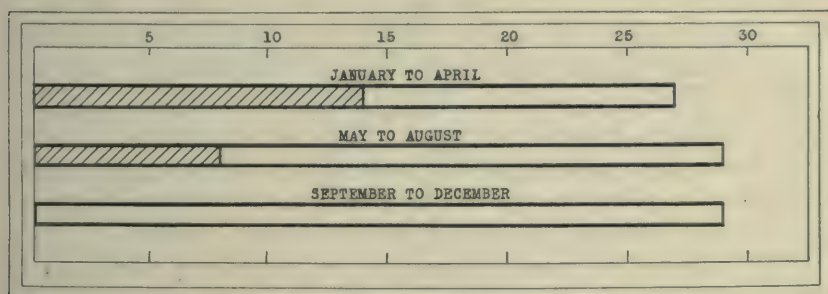


Fig. 5.—Recurrence of pellagra within three months following childbirth in different seasons. The number of instances of childbirth in each four-month period is indicated by the total length of the bar. The number of instances in which recurrence of pellagra appeared in the respective group within three months after childbirth is indicated by the shaded portion. Note that childbirth from September to December was not followed by recurrence of pellagra within three months in any instance.

the pellagrous women who bore children in the period from January to April, inclusive, most frequently suffered a recurrence of pellagra within three months. About one half of such childbirths were followed by recurrence of pellagra within this time. The childbirths in the period from May to August, inclusive, were followed by a recurrence of pellagra within three months in less than one third of the instances. On the other hand, the twenty-nine births which occurred in the period from September to December were not followed by recurrence of pellagra within three months in a single instance. It would appear, therefore, that childbirth does predispose to a recurrence of pellagra to a remarkable degree, but that this predisposing influence is not sufficient to determine recurrence of pellagra in the winter season.

## THE RELATION OF CHILDBEARING TO RECURRENCE OF PELLAGRA

In the foregoing sections it has been shown that in the population here studied, pregnancy has exercised an inhibitory influence on the recurrence of pellagra, while childbirth has predisposed to it. Inasmuch as gestation is normally accompanied by childbirth it would be of interest to know their combined influence in respect to recurrence of pellagra. If we add together the percentage of recurrences during the nine months of pregnancy, namely, 21.4, and the percentage of recurrences during the three months following childbirth, namely, 26.1, we obtain a figure, 47.5 per cent., which is an appropriate measure of the recurrence rate for twelve months for puerperal women. This is somewhat lower than the annual recurrence rate for total female pellagrins in Spartanburg County, which was 63 per cent. The figures would indicate that puerperal women are less liable to recurrence of pellagra than other women. It is impossible to decide whether this apparent difference may be due directly to the physiologic exercise of the maternal function or may be merely correlated with it on account of the greater general vigor and superior environmental conditions which the childbearing woman is likely to enjoy. In any case the difference from the general recurrence rate is not very great. It would seem, however, to justify the conclusion that childbearing is not contraindicated in pellagrins, as one might suppose if only the frequency of recurrence following childbirth were taken into account.

The influence of season appears to be particularly important in this connection. The pellagrous women who bore children in the period from January to April, inclusive, suffered recurrence of the disease during pregnancy in 5.9 per cent. of instances and the analogous group suffered recurrence in the first three months after delivery in 51.9 per cent. of instances. The approximate frequency of recurrence for the twelve months beginning with pregnancy was, therefore, the sum of these figures, or 57.8 per cent., for this seasonal group. In the group with childbirth in the period from May to August, inclusive, the recurrence rate during pregnancy was 37.5 per cent. and the recurrence rate in the three months after delivery 27.6 per cent. The sum of these, or 65.1 per cent., represents approximately the recurrence rate for the year beginning with pregnancy, for this seasonal group. For the third seasonal group, in which childbirth occurred from September to December, inclusive, the recurrence rate during pregnancy was 15.6 per cent. and in the three months subsequent to delivery it was 0.0 per cent. The recurrence rate for the twelve months in this seasonal group is, therefore, approximately 15.6 per cent.

This indicated contrast is so marked that it seems worth while to present a separate tabulation, including as definite examples only those

TABLE 12.—BEHAVIOR OF PELLAGROUS WOMEN IN RESPECT TO RECURRENCE OF PELLAGRA DURING THE TWELVE MONTHS BEGINNING WITH CONCEPTION, SUMMARIZED ACCORDING TO SEASON IN WHICH CHILDBIRTH OCCURRED

Behavior after Childbirth	White Women		Colored Women		Both Races	
	Number	Per Cent.	Number	Per Cent.	Number	Per Cent.
With childbirth occurring from January to April, inclusive						
Recurrence.....	10	58.8	1	100.0	11	61.1
No recurrence.....	7	41.2	0	0.0	7	38.9
Total definite.....	17	100.0	1	100.0	18	100.0
Uncertain.....	1	.....	0	.....	1	.....
Total.....	18	.....	1	.....	19	.....
With childbirth occurring from May to August, inclusive						
Recurrence.....	16	64.0	2	100.0	18	66.7
No recurrence.....	9	36.0	0	0.0	9	33.3
Total definite.....	25	100.0	2	100.0	27	100.0
Uncertain.....	0	.....	0	.....	0	.....
Total.....	25	.....	2	.....	27	.....
With childbirth occurring from September to December, inclusive						
Recurrence.....	5	22.7	0	0.0	5	21.7
No recurrence.....	17	77.3	1	100.0	18	78.3
Total definite.....	22	100.0	1	100.0	23	100.0
Uncertain.....	1	.....	0	.....	1	.....
Total.....	23	.....	1	.....	24	.....
With season of childbirth not definitely recorded						
Recurrence.....	1	33.3	0	.....	1	33.3
No recurrence.....	2	66.7	0	.....	2	66.7
Total definite.....	3	100.0	0	.....	3	100.0
Uncertain.....	0	.....	0	.....	0	.....
Total.....	3	.....	0	.....	3	.....



instances in which the initial attack occurred previous to the pregnancy and in which there is available definite record for both the period of gestation and the three months following delivery. The figures are summarized in Table 12. The differences between the three groups are here similar. Those women who bore children from September to December, inclusive, were only about one third as liable to recurrence during the twelve months including the whole period of pregnancy and the three months following childbirth as were the women who gave birth to children in other seasons of the year.

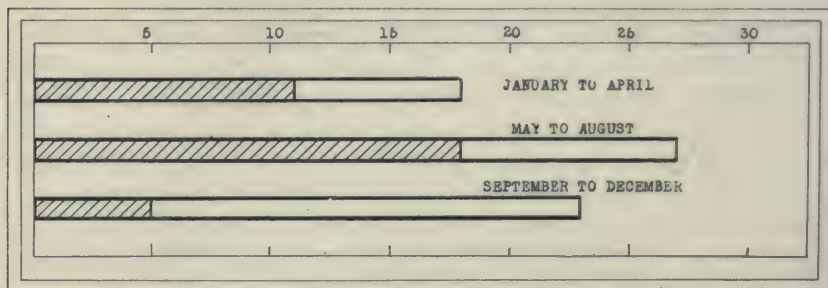


Fig. 6.—Recurrence of pellagra in childbearing women. The number of instances of childbirth in each four-month period is indicated by the total length of the bar. The number of instances in which recurrence of pellagra appeared during the twelve months, including the period of gestation and the three months following delivery, is indicated by the shaded portion. Note that only five of the twenty-three pellagrous women who gave birth to children from September to December suffered recurrence of pellagra during the twelve months.

This third group stands out in remarkable contrast to the other two groups in this respect, clearly indicating that the most favorable season for childbirth in this pellagrous population has been from September to December, inclusive. The contrast is so great that it might well be taken into account by those physicians who have pellagrous married women in their care.

#### SUMMARY

1. Twenty of the 624 initial attacks of pellagra in women in the age period 12 to 49 years, in Spartanburg County, occurred during pregnancy. This number represents 3.8 per cent. of 624 initial attacks in women of childbearing age and indicates that onset of pellagra has been relatively less frequent during pregnancy than at other times.

2. Sixteen of the 624 initial attacks, or 2.6 per cent., occurred in the month following childbirth, twenty-two, or 3.5 per cent. in the second and third months following childbirth and nineteen, or 3.0 per cent., in the fourth, fifth and sixth months following childbirth.

3. The proportion of initial attacks of pellagra in the six months

subsequent to childbirth is distinctly excessive and the indicated increased liability to the development of pellagra is greater in the earlier months following this event.

4. Among eighty-seven instances of pregnancy in pellagrous women, in only eighteen, or 20.7 per cent., did a recurrent attack of pellagra appear during the period of gestation. This frequency is distinctly lower than the recurrence rate for pellagrous females in general, namely, 63 per cent. per year, and indicates a definitely increased resistance to recurrence of pellagra during pregnancy.

5. Recurrences of pellagra during pregnancy were less frequent in the earlier months. They were usually mild in character and no deaths occurred during the period of pregnancy.

6. In those pregnancies terminating from May to August, inclusive, recurrence of pellagra was relatively most frequent. In these instances the later months of gestation coincided with the season of greatest activity of pellagra.

7. Among eighty-eight instances of childbirth in pellagrous women there were twenty-three, or 26.1 per cent., in which a recurrence of pellagra appeared within three months after this event. This recurrence rate is enormously high in comparison with the normal rate of 15.8 per cent. for three months.

8. The influence of season was evidently of great importance. Childbirth in the period from September to December, inclusive, was not followed by recurrence of pellagra within three months in a single instance. Childbirth in other seasons, on the other hand, was followed by recurrence in about one third of the instances.

9. Considering the period of gestation and the three months following it together as a whole year, we see that recurrence has not been more frequent in childbearing women than for pellagrous women in general.

10. When considered in this way, the instances of pregnancy terminating in childbirth from September to December, inclusive, showed a very low frequency of recurrence for the whole year, namely, 21.7 per cent., which is about one third of the recurrence rate for pellagrous females in general.

11. On the other hand, in those pregnancies terminating during other seasons of the year the frequency of recurrence for the year was between 60 and 70 per cent.

12. These relations are believed to be of practical significance, not only for prognosis, but also as criteria to be utilized in the treatment and management of pellagrous married women.

## FURTHER STUDIES ON TYPHOIDIN \*

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This paper records the results of further studies on typhoidin made since the publication of the original article of Gay and Force.

Since Gay and Force<sup>1</sup> have reviewed the work on the application of preparations of the typhoid organism to the skin which had been published previous to their article, we shall confine ourselves to a review of observations on typhoidin which have appeared since that date.

The original typhoidin employed by Gay and Force consisted of a ten-day culture of a single strain of *Bacillus typhosus* on glycerin broth evaporated to one-tenth volume. On account of deterioration of the preparation in this form, Gay and Claypole<sup>2</sup> precipitated the original typhoidin with twenty volumes of alcohol, filtered, washed with absolute alcohol and ether, and then dried on porcelain plates over sulphuric acid in a vacuum. With a freshly prepared suspension of this typhoidin powder in phenolated saline, equivalent in concentration to the original typhoidin, these authors were able to produce marked intradermal reactions in previously immunized rabbits, but not in controls. The reaction was characterized by the appearance within twenty-four hours of an indurated, reddened papule, which persisted at the site of inoculation for several days. Gundrum<sup>3</sup> was unable to produce positive reactions in six typhoid cases by the intradermal injection of 0.1 c.c. doses of a commercial typhoid vaccine representing a suspension of 100 million organisms to 1 c.c. Positive results were secured in ten typhoid cases out of thirteen by a similar injection of Gay-Claypole vaccine, representing a suspension of 0.1 mg. (800 million) dried and ground sensitized typhoid organisms to 1 c.c. A red maculopapule appearing at the site between six and forty-eight hours after inoculation was considered positive if it equaled in size the original wheal produced by the intradermal injection.

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\* From the Laboratory of Hygiene and the Hearst Laboratory of Pathology and Bacteriology, University of California.

1. Gay, F. P., and Force, J. N.: A Skin Reaction Indicative of Immunity Against Typhoid Fever, *THE ARCHIVES INT. MED.*, 1914, **13**, 471.

2. Gay, F. P., and Claypole, Edith J.: An Experimental Study of Prophylactic Immunization Against Typhoid Fever, *THE ARCHIVES INT. MED.*, 1914, **14**, 671.

3. Gundrum: Skin Test in Typhoid, *California State Jour. Med.*, 1915, **13**, 43.



These results are open to the objection that Gay and Claypole<sup>2</sup> have shown that marked reactions may be produced in normal persons by the intradermal injection of a suspension of sensitized vaccine representing from 0.5 mg. to 5 mg. to 1 c.c. In justice to Gundrum, however, it should be stated that he failed to produce reactions in eight controls, and eight of nine malaria patients. Pulay<sup>4</sup> was unable to produce satisfactory results with a typhoidin made from a laboratory strain over a year old. With a typhoidin prepared from a recently isolated strain he secured negative reactions in twenty-eight normal subjects. Positive reactions were obtained in six typhoid patients, thirty-five convalescents, five persons with a history of typhoid long ago, and thirty-nine persons previously vaccinated. In reading the results Pulay lays particular stress on the persistence of the maculopapule, which may be distinct in normals at the end of twenty-four hours, but never after forty-eight hours. In the immunized person, however, the papule may be distinct even on the third day. The preparation used by Pulay was original typhoidin concentrated by evaporation at 40 C. in a vacuum.

Mehler,<sup>5</sup> using original typhoidin, secured negative results in ten normal persons. Positive reactions were produced in eight of ten persons with a history of typhoid (one fifty years, one thirty years, one nineteen years, one fifteen years and again three and one-half years, and four three and one-half years previously) and negative reactions in two persons with a history of typhoid three and one-half years previous to the test. Of ten persons receiving two injections of typhoid vaccine three and one-half years previous to the test, only one gave a positive reaction, while thirteen of twenty persons receiving three injections three and one-half years previous to the test gave positive reactions.

Nichols<sup>6</sup> by cutaneous application of a typhoidin powder reached the following conclusions:

1. The test was negative in 25 per cent. of cases with a history of typhoid, while typhoid fever is supposed to give 95 per cent. immunity.
2. The test was negative in 36 per cent. of those vaccinated under four years previous to the test, while experience has shown that vaccination protects in more than 64 per cent. of cases.
3. A preparation of *B. paratyphosus* A gave 66 per cent. of positive reactions in cases giving 75 per cent. reactions to typhoidin,

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4. Pulay: Diagnostische Hautreaktion bei Typhusrekonvaleszenten, Typhuskranken und Schutzgeimpften mit Typhin nach Gay and Force, Wein. klin. Wchnschr., 1915, **28**, 1189.

5. Mehler: Oration on Medicine—Prophylaxis of Typhoid Fever, Jour. Iowa State Med. Soc., 1916, **6**, 99.

6. Nichols: Antityphoid Vaccination, Jour. Exper. Med., 1915, **22**, 780.

whereas there is no immunity to paratyphoid after typhoid immunization.

4. A positive typhoidin reaction is indicative of a history of typhoid fever or typhoid immunization, but a negative reaction is not necessarily indicative of the opposite condition.

Nichols interpreted his reactions according to the original criteria of Gay and Force; a difference in diameter between typhoidin and control spots of at least 2.5 mm. twenty-four hours after the application of the test. Had he waited for the toxic action to subside (forty-eight hours) his results with paratyphoidin might have been different. His results with typhoidin are practically identical with ours, since we obtained negative reactions in 23 per cent. of patients with history of typhoid and 36 per cent. of persons vaccinated under four years previous to the test. We are not prepared, however, to agree with his statement of the percentage of recovered typhoid patients who are protected. Sawyer<sup>7</sup> found a history of previous typhoid in 15 per cent. of cases in an epidemic occurring in Hanford, Calif., while Kelly<sup>8</sup> secured a similar history from 8.2 per cent. of cases at Taft, Calif.

Austrian and Bloomfield<sup>9</sup> employed suspensions of dried typhoidin powders derived from both a single and several strains of *B. typhosus*. These suspensions were injected intradermally in doses ranging from 0.00001 gm. to 0.02 gm. of the dried powder. The original criteria of Gay and Force were employed in interpreting the reactions, which were positive in all of sixty-six cases comprising normal persons, those previously vaccinated, and persons with a history of typhoid. In several cases they report local and general reactions comparable to those following typhoid vaccination. These results of Austrian and Bloomfield were in no way remarkable when the amount of typhoidin used is considered, together with the failure to wait for the subsidence of the irritant effect of the protein. Gay and Claypole have shown that the evaporation of 10 c.c. of typhoidin broth will yield 0.78 gm. of powder, while the evaporation of an equal amount of control broth will yield 0.5 gm. of powder. Not allowing for the increase in typhoid organisms at the expense of the broth, we may assume that any quantity of typhoidin powder contains one-third its weight of typhoid bacilli. The ordinary immunizing dose of typhoid vaccine used by us contains 0.0001 gm. of typhoid bacilli, equivalent to 0.0003 gm. of typhoidin. Doses of typhoidin then ranging from 0.00001 gm. to 0.02 gm. represent vaccinations with from one-thirtieth

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7. Sawyer: Ninety-Three Persons Infected by a Typhoid Carrier at a Public Dinner, Jour. Am. Med. Assn., 1914, **63**, 1537.

8. Kelly: Personal communication to the authors.

9. Austrian and Bloomfield: The Typhoidin Reaction, THE ARCHIVES INT. MED., 1916, **17**, 663.

to sixty-six times the ordinary dose of typhoid vaccine. A normal person might be expected to show local and general reactions to the larger dose, while a previously sensitized person might give a similar reaction to the smaller dose. Positive reactions in the previously vaccinated person could easily be confused with local reactions produced by intradermal vaccination of normal persons.

Kilgore<sup>10</sup> has suggested the use of a quotient for recording the results of cutaneous tests obtained by dividing the diameter of the allergic reaction by the diameter of its control. He regards a quotient over 1.5 as positive, basing his opinion on a number of experiments in the application of typhoidin to the scarified skin. He has pointed out the difficulty of securing uniform scarifications and the impossibility of applying to them constant amounts of the powder. Another element of unreliability in the reaction is a strong irritant effect of the powder itself, which tends to overshadow a weaker specific action. In another communication Kilgore<sup>11</sup> has shown that the higher the typhoidin quotient the greater the chance of reaction to vaccination, and that those who react least to vaccination profit most by its administration, if increase in the typhoidin quotient after vaccination be considered evidence of increased immunity. Kilgore also gives experiences with the intradermal method of administering the typhoidin test. The dose used was approximately 0.05 c.c. of a 1 to 100 suspension of dry typhoidin powder, controlled by an equivalent dose of broth powder. Observations were made at the end of twenty-four hours and results recorded in terms of the typhoidin quotient. Kilgore states that there was practically no difference in the average quotients between a group of fifty-three supposedly immune persons and a group of thirty-eight with a negative typhoid history. Some persons in this series complained of malaise and chilliness, together with an arm as sore as if a typhoid vaccination had been taken. As in the case of Austrian and Bloomfield, the results of Kilgore are not surprising when we consider the doses of typhoidin used which he himself admits were probably too large. The ordinary dose of typhoid vaccine being 0.0001 gm. (equivalent to 0.0003 gm. of typhoidin), it is evident why normal persons should be confused with those who are immune and why both groups should complain of local and general reactions on receiving an intradermal dose of unsensitized killed typhoid organisms two-thirds greater than the quantity used in typhoid vaccination. The excessive dose, together with a twenty-four-hour observation, served to effectually mask any variations in sensitiveness which might otherwise have been apparent.

10. Kilgore: The Typhoidin Quotient, *THE ARCHIVES INT. MED.*, 1916, **17**, 25.

11. Kilgore: A Comparison of Two Methods of Vaccinating Against Typhoid Fever, *THE ARCHIVES INT. MED.*, 1917, **19**, 276.



Kolmer and Berge<sup>12</sup> have made a careful comparison of the typhoidin reaction with the agglutinins, bactericidins, and complement fixing antibodies present in the serum of typhoid immune persons. The typhoidin was injected intradermally in doses ranging from 0.0005 mg. to 0.001 mg., and the resulting reaction observed in twenty-four hours. A reaction was regarded as positive "when the typhoidin site alone showed a reaction or when this site had at least twice the area of erythema and a greater edema than the control," as doubtful when the typhoidin site was one-third greater than the control, and as negative when there was "nothing more than trauma or an area of about equal degree in regard to erythema and edema." From observations on forty-two cases these authors conclude "that agglutinins are present in the blood serum of the majority of persons reacting positively in the skin test, but there is no definite relation between the two as either may be in evidence in the absence of the other." They further mention an irritant action of the typhoidin and suggest the possibility of developing a typhoidin free from toxic substances. They found positive reactions in two of ten normal persons, six of ten persons with a history of typhoid, and twelve of twenty-two previously vaccinated. It is possible that these results might have been different had the reactions been observed at the end of forty-eight hours after the irritant effect of the protein had subsided.

#### THE PREPARATION OF TYPHOIDIN

Reference has already been made to a typhoidin powder which Gay and Claypole prepared by precipitating the original typhoidin with alcohol, washing with absolute alcohol and ether, and then drying on porcelain plates over sulphuric acid in a vacuum. It was anticipated that this dried typhoidin would retain its potency for a much longer period than the original concentrated glycerin broth preparation. In an attempt to avoid the necessity of weighing a small quantity of this powder whenever a fresh suspension was needed, Dr. Claypole prepared for one of us (Force) a number of small amber vials, each containing such a quantity of the powder that the addition of 1 c.c. of phenolated saline would furnish material for a number of tests. As the powder was believed to be stable, no especial precautions were observed in storing the vials, and we were therefore surprised to discover, on examining them some days later, that the powder had become a moist, treacle-like mass. Thereafter no attempt was made to prepare a saline suspension, but the powder was stored in a large vial in a desiccator, and applied directly to the scarified arm by means of the

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12. Kolmer and Berge: The Relation of the Typhoidin Skin Reaction to Immunity in Typhoid, *Jour. Immunol*, 1916, **1**, 409.

chisel used in making the abrasion. The obvious tendency of the powder to absorb moisture, however, raised the question as to the possible hydrolyzing effect of the residual moisture during the rather slow process of drying over sulphuric acid. Studies were therefore made by one of us (Stevens) with a view to developing a method for the rapid dehydrating of the typhoidin powder to a constant weight, experience with other proteins having shown that a rapidly dehydrated preparation is extremely stable.

The method of preparation finally adopted was a modification of the technic of Gay and Claypole. Ten flasks, each containing 25 c.c. of meat infusion broth (1 per cent. peptone), were inoculated with ten different strains of *B. typhosus*, and incubated for ten days. The ten cultures were then poured into a large glazed porcelain dish and the mixture was evaporated for sixteen hours over an acetone bath to one-tenth the original volume. This concentrated polyvalent culture was then transferred to a 500 c.c. stoppered cylinder, thirty volumes of 95 per cent. alcohol were added, the mixture thoroughly shaken and allowed to stand for twenty-four hours. At the end of this period the supernatant fluid was drawn off by means of a pipet attached to a filter pump, the precipitate washed in 95 per cent. alcohol, and allowed to stand until entirely settled. The alcohol was again drawn off and the precipitate washed twice with absolute alcohol, and the precipitate allowed completely to settle in each instance before removing the supernatant fluid. After the removal of the alcohol the precipitate was shaken up with absolute ether, allowed to settle, again shaken, the suspension poured onto a hard filter paper (C. S. & S. 575), and the precipitate again washed with absolute ether, being careful to keep it covered with a watch glass while draining. The covered funnel containing the precipitate was immediately placed in an oven containing an open dish of sulphuric acid, and kept at a temperature of 40 degrees C. for thirty minutes. At the end of this time the watch glass was removed from the filter and the open filter left in the oven for eighteen hours. The filter paper was then removed from the funnel, opened out, and left in the oven until the precipitate crumbled readily. It was then transferred to an agate mortar, ground by hand, placed in a dark-colored, glass-stoppered vial, weighed, and exposed in a desiccator over sulphuric acid. When the powder has dried to constant weight the stopper may be inserted and sealed with paraffin.

The following comments on this method of preparation may perhaps be in order: (1) The different strains of *B. typhosus* used in making the polyvalent typhoidin were selected to represent the various

antigenic properties described by Hooker.<sup>13</sup> (2) The growth in each of the ten flasks was determined to be a pure culture before the strains were mixed. (3) As soon as the suspension of typhoidin in absolute ether is poured onto the filter it should be covered with the watch glass and kept covered, except when more ether is poured on for washing. A few seconds' exposure to the air at this stage will convert the precipitate into a gumlike mass, whereas the particles of typhoidin adhering to the mortar after the grinding show no tendency to deliquescence even after forty-eight hours' exposure. (4) In the absence of a vacuum chamber with thermostatic control, the ordinary gas oven may be used with hot bricks as radiators. The gas flame should be shut off before placing the filter in the oven. (5) Given the ten days' culture, three days should be sufficient for all of the steps, beginning with the concentration of the culture and ending with the exposure of the dry powder in the desiccator. The powder should be dried to constant weight in four days at the most. A period of eight weeks has been found necessary in some instances to prepare dry typhoidin according to the method of Gay and Claypole.

#### THE TYPHOIDIN TEST AND ITS INTERPRETATION

We have long recognized that the application of typhoidin to the scarified skin is open to the objections so well described by Kilgore. In entering on this series of studies, therefore, we determined to employ the more accurate method of intradermal injection.

Suspensions of typhoidin and its corresponding bouillon control were made in phenolated saline (0.5 per cent. phenol), so that 0.1 c.c. would contain 1 mg., 0.1 mg., or 0.01 mg. of the dried powder in each instance. Three rabbits which had been immunized against *B. typhosus* one year previously and reimmunized six weeks before the test were shaved on the back. On the following day they were inoculated intradermally with 0.1 c.c. of each suspension. Three normal rabbits served as controls. The results of this experiment are shown in Table 1. It will be seen that the dose of 1 mg. of typhoidin is unduly toxic, while the dose of 0.01 mg. did not produce a reaction in the immune animal distinguishable from the reaction in the normal. The medium and low doses were then given to L. R. T., a student, who had been vaccinated within the year. Reference to Table 1 will show that a dilution too high to give definite results in immune rabbits produced a distinct reaction in a previously vaccinated person.

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13. Hooker: A Study of the Antigenic Properties of Different Strains of *B. typhosus*: Preliminary Communication, Proc. Soc. Exper. Biol. and Med., 1916, **13**, 139.



Von Pirquet,<sup>14</sup> Force and Beckwith,<sup>15</sup> and Kolmer and Moshage<sup>16</sup> have shown that irritation due to trauma is reduced to a minimum by intradermal inoculation of 0.05 c.c. of fluid. We therefore finally adopted a dose of 0.005 mg. of typhoidin suspended in 0.05 c.c. of phenolated saline, representing a dilution of 1 to 10,000.

In interpreting the results of the typhoidin test, we have established the following criteria:

1. A positive reaction is indicated by the presence, forty-eight hours after the application of the test, of a maculopapule, with definite erythema measuring at least 5 mm. in one diameter.

TABLE 1.—TESTS TO DETERMINE THE DOSAGE OF TYPHOIDIN

Subject	Dose, Mg.	Reaction First Day		Reaction Second Day		Result
		Typhoidin	Control	Typhoidin	Control	
Rabbit						
1. Immune.....	1	((28.3)	7.6	(19.5)	10.9	Positive
2. Normal.....	1	20.5	7.1	22.9	0	Negative
3. Immune.....	0.1	((17.5)	5	( 9.9)	0	Positive
4. Normal.....	0.1	16.8	0	12.8	0	Negative
5. Immune.....	0.01	(16	7	5.6	2.8	Negative
6. Normal.....	0.01	16	11.8	6.5	0	Negative
Human						
L. R. T., vac. 1915...	0.1	((65.3 ((11.3)	21	((99.8 ((14)	0	Positive
	0.01	(29.9)	25	((76.1 (( 9.4)	0	Positive

2. A doubtful reaction is indicated by the absence of either induration or erythema at the end of forty-eight hours.

3. A negative reaction is indicated by the absence of both induration and erythema at the end of forty-eight hours.

Von Pirquet has shown that intradermal inoculations of vaccine lymph produce, in previously vaccinated persons, marked papules which increase in size to the forty-eighth hour and persist even to the seventy-second hour. A good reaction might be produced by this method when an equal amount applied to the scarified skin failed to

14. Von Pirquet: Ueber die verschiedenen Formen des allergischen Reaction bei der Devaccination, Ztschr. f. Immunitätsforsch. u. exper. Therap., Orig., 1911, **10**, 1.

15. Force and Beckwith: A Laboratory Method for the Diagnosis of Smallpox, Jour. Am. Med. Assn., 1915, **65**, 588.

16. Kolmer and Moshage: A Note on the Occurrence of Pseudoreactions on the Skin, with Special Reference to the Schick Toxin Test, Jour. Am. Med. Assn., 1915, **65**, 144.

produce results. Haring and Bell<sup>17</sup> in observing the intradermal tuberculin reaction consider positive any induration at the point of inoculation larger than the head of a parlor match, increasing in size to the forty-eighth hour and persisting to the seventy-second hour. Gay and Claypole<sup>2</sup> describe the typhoidin reaction on immune rabbits as a red areola with a nodule measuring from 2 to 5 mm. which persists several days after the disappearance of the areola. Force and Beckwith<sup>15</sup> have shown that smallpox vesicle contents inoculated intradermally into previously vaccinated rabbits will produce a red areola and infiltration appearing within twenty-four hours, but which may not reach its maximum until forty-eight hours. Kolmer and Moshage<sup>16</sup> advise the interpretation of diphtheria toxin intradermal reactions on a forty-eight rather than a twenty-four hour basis, though this reaction is not allergic in the sense of the so-called pseudoreaction to diphtheria protein. Levinson<sup>18</sup> would regard no diphtheria toxin test positive unless there was induration measuring at least 5 mm. by 2 mm. and persisting five days with change of color. Weil,<sup>19</sup> in his intradermal tests to detect the presence of pneumonia antibodies, regards the reaction positive when there is an area of erythema and a papule persisting forty-eight hours or more. Combe,<sup>20</sup> in his intradermal tuberculin tests in children, measures the papule present forty-eight hours after the injection of 0.1 mg. of tuberculin. Zingher<sup>21</sup> describes the pseudoreactions obtained by the intradermal injection of diphtheria toxin as anaphylactic responses of the tissue cells to the protein substances of the autolyzed diphtheria bacilli in the toxic broth. The reaction appears early, within from six to eighteen hours, reaches its height in from thirty-six to forty-eight hours and disappears on the third or fourth day, leaving no pigmentation. It is characterized by a small central area of dusky red infiltration, with a secondary areola which shades off into the surrounding skin.

In all of these descriptions of skin tests there seems to be a disposition to recognize the persistence of a well-marked papule to the forty-eighth hour and erythema as more satisfactory criteria for positive reactions than any comparison of allergin with control. In fact in our later studies we have discontinued the use of the control

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17. Haring and Bell: The Intradermal Test for Tuberculosis in Cattle and Hogs, Univ. Calif. Pub. Ag. Bull., No. 243, 1914.

18. Levinson: The Value and Limitations of the Schick Diphtheria Reaction, Illinois Med. Jour., 1915, **28**, 405.

19. Weil: Note on a Skin Reaction in Pneumonia, Jour. Exper. Med., 1916, **23**, 11.

20. Combe: Diagnosis of Tuberculosis in Infants, Le Nourrisson, January, 1916, **4**, 73.

21. Zingher: Methods of Using Diphtheria Toxin in the Schick Test and of Controlling the Reaction, Am. Jour. Dis. Child., 1916, **11**, 269.

entirely. Typhoidin represents something more complicated than typhoid bacilli plus peptone broth. At the end of ten days of growth and digestion the organisms have seriously affected the peptone and beef proteins. It follows, therefore, that a powder produced by the evaporation of peptone broth cannot truly serve as a control to typhoidin. Since many persons give marked reactions to peptone broth, it would seem that any attempt to interpret the reaction in terms of the difference between the typhoidin spot and a broth control will lead to much misinterpretation of results.

Our determination to abandon the use of a broth control is in line with the procedure of Zingher,<sup>22</sup> who, in the routine administration of the diphtheria toxin test, has substituted for the control broth a heated toxin, which is administered intradermally to those persons giving a doubtful reaction to the diphtheria toxin. Heating destroys the irritant action of the toxin and any reaction to this product would represent the uncomplicated allergic action of autolyzed diphtheria organisms, that is, the true pseudoreaction. The heated toxin then becomes, for the purpose of this reaction, an actual control to the unheated toxin. Zingher also has observed that persons giving the pseudoreaction, that is, presumably sensitized to diphtheria protein, were much more susceptible to the local action of antitoxin or immunizing injections of toxin-antitoxin. Weaver and Rappaport<sup>23</sup> have noted that persons giving an intradermal pseudoreaction to diphtheria toxin are apt to give a similar reaction to a control of toxin-antitoxin. These authors charge this result to the effect of horse serum, but it is also readily explained on the basis of allergic effects of autolyzed diphtheria organisms. Since Bessau and Schwenke<sup>24</sup> have been unable to remove the allergic effect of autolyzed diphtheria organisms even by boiling diphtheria toxin, it follows that the question of a true control to typhoidin or other cutaneous allergins is still an open one.

#### SPECIFICITY OF THE TYPHOIDIN REACTION

We have suggested criteria for the interpretation of the typhoidin reaction independent of so-called controls. There remains still the question of specificity. In other words, is the typhoidin reaction due to a toxic effect of the inoculated protein on the tissues, or, even granting such an effect, is there in addition a specific reaction in persons sensitized to typhoid protein? If there is such a reaction how may it be unmasked from the stronger toxic influence?

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22. Zingher: The Pseudoreaction in the Schick Test and Its Control, *Jour. Am. Med. Assn.*, 1916, **66**, 1617.

23. Weaver and Rappaport: Further Observations on the Schick Test for Diphtheria Immunity, *Jour. Am. Med. Assn.*, 1916, **66**, 1448.

24. Bessau and Schwenke: Ueber de lokale Diphtherie-Bouillon-Reaktion beim Menschen, *Monatsch. f. Kinderh.*, 1916, **13**, 397.



Stokes<sup>25</sup> holds that many skin reactions are not specific in the sense that they are the results of antigen-antibody activity, but rather the results of the activity of an anaphylatoxin which will produce focal inflammation as the result of the action of the subject's enzymes on his own tissues. This anaphylatoxin is a product of the action of a protease released through parenteral introduction of antiferment adsorbents. On the other hand, Bronfenbrenner<sup>26</sup> claims that more than mechanical action of antiferment adsorbents is involved in these reactions, since there is an element of specificity, not in the ferment, but in the mechanism of its activation. The combination of a specific serum with its corresponding antigen is followed by a change in the

TABLE 2.—DETAILS OF—

Initials	Class	Date of Test	Preparation			
			MAc	MAI	PAc	PAI
M. C. ....	Normal.....	2/23/16	#	...	...	...
H. D. ....	Normal.....	2/23/16	#	...	...	...
M. S. ....	Normal.....	4/ 3/16	...	#	...	...
H. I. S. ....	History of Typhoid— 1906.....	3/22/16	...	...	...	...
F. B. ....	1906.....	3/15/16	#	...	...	...
R. L. S. ....	Vaccinated 3/31/14, 3 doses; 3/3/15, 2 doses; typhoid 5/25/15.....	4/26/16	...	...	...	#
D. G. ....	Previously Vaccinated— 2/ 7/14 (3) X..... 3/15/16 (3) Y.....	3/ 1/16 4/12/16	# ...	... ...	... #	... ...
J. M. ....	5/12/14 (3) X..... 3/ 3/16 (2) X.....	2/25/16 5/ 1/16	# ...	... ...	... ...	... #
C. G. P. ....	9/24/13 (2) X..... 4/ 3/16 (3) X.....	3/27/16 4/24/16	# ...	... ...	... ...	... #

# From February to May, 1916, inclusive.

degree of dispersion of serum colloids, which is in turn followed by the activation of the nonspecific ferment.

While we must admit that parenteral administration of foreign proteins gives rise to certain nonspecific responses, we hope to show in the case of typhoidin that these general reactions tend to subside by the forty-eighth hour; and further, that these reactions are proportional to the concentration of the suspension of typhoidin powder, so that the specific reaction appears to be unmasked by using small doses of the antigen.

25. Stokes: Studies on Intradermal Sensitizations, II. An Intradermal Reaction to Agar and an Interpretation of Intradermal Reactions, *Jour. Infect. Dis.*, 1916, **18**, 415.

26. Bronfenbrenner: Specific Parenteral Digestion and Its Relation to Phenomena of Immunity and Anaphylaxis, *Jour. Lab. and Clin. Med.*, 1916, **1**, 573.

## EXPLANATION OF THE TABLES

The accompanying tables, 2, 3 and 4, show the results of typhoidin tests on a group of faculty and students of the University of California.

A uniform dose of 0.05 c.c. of a suspension in phenolated (0.5 per cent. phenol) saline of 0.005 mg. of typhoidin was injected intradermally in each instance. Any injection failing to produce a white wheal with depressions marking the hair follicles was discounted in the estimation of results, but there was no attempt to produce wheals of constant diameter.

## —NINE TYPHOIDIN TESTS

Reaction First Day			Reaction Second Day		Reaction Fifth Day		Result
Typhoidin		Control	Typhoidin	Control	Typhoidin	Control	
26.8	5.8	6.5	4.1	4	5.9	4.5	—
(28.7		4.6	5.2	4.6	3	2.9	—
17.8	3.9	10 5	3.6	3.7	3.1	3.1	—
46.4	(( 8.3)	7.5	56.5 ((12.8)	10	15 (( 5.1)	3.6	+
(27.3	(12.5)	8.9	.....	...	(10 )	0	+
10.3		.....	( 6.5	...	5 )	...	±
11.5	( 6.1)	6.1	5	4.9	5.7	3.8	—
.....	.....	.....	(23 ((13	...	(( 5.5)	...	+
37.3	( 7.4	3.5	7.8	2.9	0	0	—
(31.5	(10.6)	.....	24.3 ( 6.4)	...	.....	0	+
(31.6	( 7.3)	5)	5.8	3.5	.....	...	—
18.6	( 5	7	(( 9.5)	0	( 5 )	0	+

Table 2 gives the details of nine typhoidin tests and is presented in order to show the methods employed in recording the results obtained.

The preparations of typhoidin employed were a monovalent strain precipitated with acetone (MAc), a monovalent strain precipitated with alcohol (MAI), a polyvalent strain precipitated with acetone (PAc), and a polyvalent strain precipitated with alcohol (PAI), which was rapidly dehydrated.

In case the person had been previously vaccinated, the date of the last dose of vaccine is given, together with a figure, in parenthesis, indicating the number of doses of vaccine and a letter indicating the type of vaccine used. The types of vaccine which had been employed in the vaccination of this series were U. S. Army (A), commercial (C), Gay-Claypole (X), and a vaccine prepared by the same technic as the latter but unsensitized (Y).

TABLE 3.—SUMMARY OF TYPHOIDIN TESTS

Class	Positive	Doubtful	Negative	Negative, %
Normals.....	1*	..	17	94
History of Typhoid:				
1899-1904.....	5	..	5†	50
1905-1910.....	9	..	1	10
1911-1916.....	5‡	1§	..	0
Previously Vaccinated:				
1913.....	25	1	26	50
1914.....	14	2	13	45
1915.....	7	1	3	27
1916#.....	37	7	12	21

\* For a history of the case of A. G. see matter under the head "Comment" in the text.

† Four of these gave questionable typhoid histories.

‡ One of these gave a questionable typhoid history in 1915, but had been vaccinated in 1912.

§ For a history of the case of R. L. S. see the case report under the head "Comment" in the text.

TABLE 4.—REACTION TO VACCINATION (OR REVACCINATION) OF PERSONS PREVIOUSLY RECEIVING TYPHOIDIN TESTS

Class	Result of Test	Persons Reporting after Each Inoculation			Local Reaction						General Reaction					
					Slight			Severe			Slight			Severe		
		1st	2d	3d	1st	2d	3d	1st	2d	3d	1st	2d	3d	1st	2d	3d
Normal.....	Untested	236	223	105	88	74	30	40	43	9	87	90	19	9	10	2
	Positive	1	....	....	..	..	..	1	..	..	..	..	..	1		
	Negative	9	8	3	3	3	..	1	1	1	2					
Previously vaccinated...	Positive	3	1	....	1	..	..	2	..	..	1	..	..	2	1	
	Doubtful	8	3	1	1	..	..	3	1	1	1	..	..	2	1	
	Negative	43	34	21	15	6	3	9	5	..	15	16	5	1	1	
History of typhoid.....	Negative	5	2	1	..	..	..	1	..	1	..	..	1	1	1	

The measurements of the reactions were made with calipers in the longest diameter and recorded in millimeters. The numbers at the left of each column of measurements refer to the areolae, the numbers at the right to the papules. A well-marked areola is designated by the sign ( placed in front of the measurement. A well-marked papule is indicated by the sign ) placed after the measurement. If the erythema of the papule is well marked, the sign ( is placed in front of the measurement. A double sign indicated great intensity of the reaction. For example: The record 25.4 ( (10.6) would indicate an areola of 25.4 mm. not very well marked, with a central papule of 10.6 mm. well marked and very red. Positive reactions are indicated by the sign +, doubtful by the sign ±, and negative by the sign —.



## COMMENT

We are, of course, aware of the unreliability of personal statements regarding the presence or absence of a history of typhoid fever. The experience of Sawyer<sup>7</sup> in the Hanford epidemic previously mentioned, has shown the possibility of typhoid fever occurring after a very short incubation period or with slight or indefinite symptoms. The normal persons in our series (Table 3), therefore, have been chosen, with one exception, after elimination of suspicious infections or possible exposure to typhoid fever. The exception noted, A. G., was a physician who, during three years residence in a hospital, was constantly exposed to the disease, though having no memory of an infection. It is interesting to note that A. G. furnished the only positive reaction in our series of normal persons, and our suspicion that he had become sensitized to typhoid protein was further strengthened by a severe local and general reaction following a subsequent dose of typhoid vaccine.

The selection of a group of persons with a history of typhoid is a much more difficult task. The retention of a person in our series (Table 3) was conditioned on a history of one or more attacks of continued fever which had been called typhoid by the attending physician. Whenever in spite of a diagnosis of typhoid the patient's account of the disease contained an element of doubt, we have regarded the history as questionable. It will be seen that on a time basis these doubtful cases tend to group themselves at the beginning of the series before the use of the agglutination test became general.

Table 3 also shows the results of the test as applied to previously vaccinated persons and further classifies these results on a time basis. It will be seen that the percentage of negative reactions grows smaller as the time elapsing from vaccination to test grows shorter. The fact that 21 per cent. of persons vaccinated during the period from February to May, 1916, inclusive, failed to show positive reactions would indicate that the routine three doses of typhoid vaccine are not always enough to sensitize or immunize a person. On the other hand, ten out of thirteen persons showing negative skin reactions before vaccination (or revaccination) gave positive reactions after such vaccination. Of the three persons failing to give a positive reaction after retest, one had received three doses of vaccine within the two months preceding the test, one six doses in the year preceding, and one six doses in the two years preceding.

The insensitiveness of certain persons to typhoid protein is well illustrated by the following case:

R. L. S., a student, after six doses of typhoid vaccine failed to give a positive skin reaction. One month after her last test (eleven weeks after her

last dose of vaccine) she developed typhoid fever, confirmed by blood culture and agglutination tests. She was treated with intravenous injections of Gay-Claypole vaccine and on recovery failed to give more than a very doubtful skin reaction.

This case further illustrates that sensitiveness bears a direct relation to protection.

Table 4 gives a summary of the local and general reactions to vaccination in persons previously receiving typhoidin tests. The reactions are arranged on the basis of response to the typhoidin test. In general it will be seen that the reactions of normal persons (both untested and negative to the skin test) differ very little from the reactions of previously vaccinated persons showing negative typhoidin tests. The revaccination of persons showing complete or partial sensitization, however, was attended by an increase in the severity of the reactions.

It is our growing conviction that typhoid immunity is a much less stable condition than has been generally supposed.<sup>27</sup> Whether, as suggested by Wassermann and Sommerfeld,<sup>28</sup> the reduced susceptibility produced by vaccination is liable to fluctuate under the influence of undernourishment, secondary infection and poor hygienic conditions; or the routine administration of three doses of typhoid vaccine is not a safe rule for every person, the fact is that many persons remain insensitive to typhoid protein after vaccination or lose their sensitiveness in increasing numbers as time elapses. In the words of Gay,<sup>28</sup> "in the case of human beings that have been vaccinated against typhoid fever we have no assurance that they are really protected and still less the assurance as to how long the protection lasts."

Pending further information on the duration of immunity, we have made a routine practice of advising the students of this university to return for a typhoidin test two years after vaccination. Students now being vaccinated (or revaccinated) are advised to return in three weeks for a typhoidin test. All persons showing a negative

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27. Reference to our Table 3 will show that we secured negative typhoidin reactions in 50 per cent. of persons who had been vaccinated against typhoid three years previously; in 45 per cent. vaccinated two years previously; and in 27 per cent., one year previously. Lescohier (*Duration of Immunity Following Smallpox Vaccination*, Jour. Am. Med. Assn., 1913, **61**, 487) has confirmed observations of Kitasato which show that 47 per cent. (48 per cent. Lescohier) of persons successfully vaccinated against smallpox may be successfully revaccinated after three years; 33 per cent. (33 per cent. Lescohier) of persons after two years, and 14 per cent. (28 per cent. Lescohier) after one year. The diminution of immunity following smallpox vaccination, as indicated by susceptibility to revaccination, shows a striking similarity in point of time to the diminution of cutaneous hypersensitiveness to typhoid protein.

28. Wassermann and Sommerfeld: *Experimentelle Untersuchungen über die Wirksamkeit der Typhus und Cholera-schutzimpfung*, Med. Klin., 1915.

28. Gay: *New Uses of Specific Skin Tests in Certain of the Infectious Diseases*, Am. Jour. Med. Sc., 1915, **149**, 157.



reaction are revaccinated, for even if the cutaneous sensitiveness to typhoid protein is of shorter duration than typhoid immunity, revaccination on the basis of a negative typhoidin reaction gives the student a safer margin of protection.

#### SUMMARY AND CONCLUSIONS

1. A stable preparation of typhoidin may be rapidly prepared by precipitating a concentrated broth culture of *B. typhosus* with 95 per cent. alcohol, and subsequent dehydration with absolute alcohol and absolute ether.

2. Definite reactions persisting forty-eight hours were produced in human beings by the intradermal injection of 0.05 c.c. of a 1 in 10,000 suspension of typhoidin in phenolated saline. In order to produce a similar reaction in rabbits, a dose of 0.1 c.c. of a 1 in 1,000 suspension of typhoidin was necessary.

3. It is imperative that no account be taken of the appearance of the reaction at the end of twenty-four hours. A positive typhoidin reaction is indicated by the presence, forty-eight hours after the test, of a well-defined erythematous papule at least 5 mm. in diameter. Out of 108 positive reactions, the forty-eight hour papule measured 10 mm. or over in twenty-six instances; the average measurement of 108 papules was 8.4 mm.

4. Out of eighteen normal persons, seventeen gave negative reactions; out of twenty-six persons with a history of typhoid, nineteen gave positive reactions, one gave a doubtful, and six persons (four with questionable typhoid histories) gave negative reactions. Out of 152 persons previously vaccinated against typhoid, twelve of fifty-six vaccinated during 1916, three of eleven vaccinated during 1915, thirteen of twenty-nine vaccinated during 1914, and twenty-six of fifty-two vaccinated during 1913 gave negative reactions (Table 3).

5. Ten of thirteen persons showing a negative reaction to the skin test before vaccination (or revaccination) gave positive reactions after vaccination (or revaccination).

6. The revaccination of previously vaccinated persons showing negative skin reactions produced local or general reactions no greater in intensity than the vaccination of normal persons, and much less severe than the reactions produced by the revaccination of persons showing positive skin reactions (Table 4).

7. The routine administration of three doses of typhoid vaccine is in many cases not sufficient to produce sensitization to typhoid protein, and presumptively, therefore, protection against typhoid fever.



8. Even granting that typhoid immunity is of longer duration than cutaneous sensitiveness to typhoid protein, the disappearance of this sensitiveness furnishes an indication for revaccination of the person, and still allows a margin of safety within the as yet indefinite limits of typhoid immunity. There is as yet no evidence that a positive typhoidin test is not indicative of protection against typhoid fever. In two instances at least, in our experience, a negative typhoidin test after vaccination was followed by typhoid infection. We feel justified, then, in proceeding on the original assumption of Gay and Force that this test may be used as a measure of protection against typhoid.

# ON THE RELIABILITY OF THE WASSERMANN REACTION

A STUDY OF THE SOURCES OF ERROR AND AN ATTEMPT TO  
STANDARDIZE THE TECHNIC \*

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In the last few years several articles<sup>1</sup> have appeared reporting inconsistencies between the results of different laboratories on the Wassermann reactions of identical specimens of blood and tending to throw doubt on the diagnostic value of the Wassermann reaction. To show that this state of affairs was becoming one of great practical significance one need only refer to the fact that in 1915 a committee was summoned by Dr. Haven Emerson, then deputy commissioner (now commissioner) of health of New York City, to consider the whole question of the reliability of the Wassermann reaction, and to attempt to standardize the technic. One of the particular reasons for the formation of the committee was the use of the Wassermann reaction by the department of health in the medicolegal examination of certain persons, such as food handlers. This committee, composed of serologists in charge of Wassermann reactions in large New York institutions, held many meetings and obtained some interesting results, but unfortunately its work was not completed. The chief difficulty encountered was that although each of the workers on the committee was doing a large number of tests, and although each of them felt that he was doing the Wassermann reaction according to classic and well-recognized principles, hardly any two of the dozen members of the committee agreed precisely in the technic for any single step in the reaction.

It should be stated at once that with the exception of Dr. Coca, who has expressed his opinions elsewhere,<sup>2</sup> the members of the committee were unanimously of the belief that in the vast majority of cases the Wassermann reaction is reliable and (in our climate, with exceptions

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1. Wolbarst: New York Med. Jour., 1913, **97**, 378; Interstate Med. Jour., 1915, **19**, No. 2. Pusey: Jour. Am. Med. Assn., 1913, **61**, 921. Stone: New York Med. Jour., 1914, **99**, 1242. Gottheil: Progressive Medicine, Sept. 1, 1914, p. 161. Coca: Bull. Johns Hopkins Hosp., 1916, **27**, 155. Ohle and MacKinney: Jour. Am. Med. Assn., 1915, **65**, 863.

2. Coca: Jour. Immunol., 1916, **1**, 484.

well known and easily excluded) diagnostic of syphilis; that most of the relatively few instances in which contradictory reports were received from different laboratories were cases of actual syphilis having very weak reactions detectable by certain technical methods, but not by others; and that when all possible precautions are taken false positive reactions (that is definite positives, complete or almost complete fixations) are avoidable.

As a member of the committee, I took on myself the task of searching for the origins of some of the variations in technic. I found that different writers on the subject had varied among themselves as widely as did the individual workers in New York City. In fact in trying to get at the truth about such important questions as the superiority of one or another antigen, these variations in technic of different workers offered great obstacles. It therefore seemed necessary that before any serious comparative work on these questions could be undertaken, certain standards of technic would have to be adopted.

The review of the literature attracted me to the study of the sources of error in the reaction. And in the present article it is proposed to present the results of experiments on this subject, as well as to review critically the principal variations from the original technic described by Wassermann. Only those methods will be considered which do not depart very widely from the original Wassermann reaction. Methods of serodiagnosis involving considerable novelty, such as that of Noguchi, of Bauer, of Stern, and of Hecht and Weinberg, however valuable in themselves, will not be discussed. The committee called together by the New York board of health failed to come to an agreement, not so much because of radical differences of opinion, as because it lacked a definite plan for attacking the problem. It is one of the objects of the present study to offer such a plan for future work.

To many readers some of the considerations detailed below may seem so minute as not to warrant serious consideration. Nevertheless, the fact remains that while different laboratory workers can almost invariably detect the strongly positive Wassermann reactions and agree in the vast majority of the definitely normal cases, their results in detecting weakly positive reactions with regularity sufficient to have diagnostic value disagree widely. These irregularities are not accidents, but are due to variations in minor points of technic which are usually considered of little importance.

One of the most discouraging features about any attempt to standardize the reaction or to compare results (such as the attempt made by the committee referred to) is the dogmatic assurance of many workers that the particular method that they use (though they never have tried any other) gives the best results. It is hoped that the pres-



ent paper will help to a more catholic attitude. As a result of personal acquaintance with a large number of workers the author is convinced that the man is more important than the method, and that some workers obtain good results with methods in themselves inferior, because the worker is intimately informed of the peculiarities and limitations of the method he uses. Not a few of the workers, indeed, follow a technic that can be described only as "rule of thumb" and that does not admit of accurate comparison or description, because it depends entirely on the experience of the worker. In fact some who have published descriptions of their methods introduce into their actual work so many personal features that it would be impossible for another worker to follow their printed directions and obtain identical results. Such methods have no advantage and should be dropped for those which admit of precise description and of successful repetition from the description by any competent worker.

The present paper will follow the plan of discussing each source of error and each point on which different workers vary under a separate heading.

#### THE TOTAL VOLUME OF THE REACTION

Wassermann and his immediate followers, such as Citron and Meier, used each of the five reagents entering into the reaction (patient's serum, antigen, complement, cell emulsion and amboceptor) in such a dilution that 1 c.c. of each was employed and the total volume at the end of the reaction was 5 c.c. For the sake of economy subsequent workers have used smaller amounts, such as one half, one quarter, or one tenth of all the reagents involved, so that the volumes of their reactions were 2.5 c.c., 1.25 c.c., or 0.5 c.c. In the present paper for purposes of comparison the quantities given by all the writers will be translated into terms of the original 5 c.c. volume.

Whatever particular volume is decided on by the worker must be adhered to uniformly in all tubes throughout titrations and tests; otherwise fallacious results are easily obtained, because the activity of complement depends on its concentration and not on the total amount present. This is a point of some importance which does not always receive the attention it deserves. It is suggested that future writers can contribute greatly to the ease of study of the subject if they will describe all their work in terms of the original Wassermann volume, simply stating that the actual quantities used were one-half, one-fourth or one-tenth of those given.

It will be necessary to exclude from consideration in this present study the work of a few authors who, without any special reason, use such arbitrary quantities of the various reagents that their results cannot be compared with those of any one else. It is unfortunate too that many of those who have written on the subject, particularly those who have written statistical or clinical articles, have failed to describe their technic or have described it so imperfectly that their work has little value.

## METHODS OF KEEPING THE HEMOLYTIC SYSTEM CONSTANT

The correct adjustment of the hemolytic system is the crucial factor in the technic of the reaction. The strength of the complement in guinea-pig serum is the most variable factor which enters into the Wassermann reaction. If it were not for this it would be easily possible to give definite rules for the quantities of all the reagents. Before discussing the various methods which have been used for adjusting the amounts of the hemolytic system, it will be worth while to review briefly a few of the fundamental facts of their quantitative relationships.

Morgenroth and Sachs<sup>3</sup> showed that within certain limits the amounts of complement and of hemolytic amboceptor needed to lake a given amount of red cells were in inverse proportions, that is to say, the more complement used, the less amboceptor was needed and vice versa. However, a limit is presently reached in each direction, a smallest amount of amboceptor with amounts smaller than which the addition of further complement does not produce complete laking, and a smallest amount of complement beyond which further increases of amboceptor will not produce complete laking. These smallest hemolytic amounts it was proposed by Bordet to call units of amboceptor and complement, with the idea that in any given serum the volumes which contain these units contain actually equal quantities of the active ingredients.

Unfortunately the terms unit of complement and unit of amboceptor have come to be used in several senses, so that they have practically lost their original significance. Most writers have titrated complement with some arbitrary amount of amboceptor, or amboceptor with some arbitrary amount of complement, and have called the minimal hemolytic dose in each case a unit of the respective substance. Probably the most generally accepted definitions are given by Noguchi;<sup>4</sup> the unit of amboceptor is the smallest amount that will completely lake the standard amount of cells in the standard volume of fluid in one hour at 37 C. (in the water bath) in the presence of an excess of complement; and the unit of complement is the least amount of complement which will give complete laking with one such unit of amboceptor. In the present article the term unit will be retained, but it will be specified in each instance what kind of unit is meant.

Certain other factors, namely, the duration and mode (whether in water bath or air thermostat) of incubation, also greatly affect the results, but for the sake of clearness a consideration of these factors will be deferred. Likewise the various technical factors which are believed to affect the strength of complement (such as the mode of obtaining blood, whether the guinea-pigs are etherized or not, the time which the blood is allowed to stand on the clot, etc.) will not be considered here, as the fundamental fact to be faced is that quite without these factors the strength of the complement of different guinea-pigs varies widely and the problem to be solved is how to make allowance for this variation.

Wassermann used a fixed dose (namely, 0.1 c.c.) of fresh guinea-pig serum as complement, 1 c.c. of 5 per cent. sheep cells, and two units of amboceptor. By two units of amboceptor he meant twice that amount of amboceptor which gave complete laking of the amount of cells stated, in one hour, in the air thermostat at 37 C., with the fixed dose of complement. The dose of complement was chosen because it was believed to be an excess, that is, to represent at

3. Morgenroth and Sachs: *Berl. klin. Wchnschr.*, 1902, No. 35, p. 817.

4. Noguchi: *Serum Diagnosis of Syphilis*.



least two Bordet units (twice the least amount of complement which would give complete laking with a very large excess of amboceptor). Wassermann made allowance for the natural variation in strength of guinea-pig complement by varying the amount of amboceptor, the two units being redetermined by a fresh amboceptor titration each time the tests were made. This method has been adhered to by Citron and many other subsequent workers.

The question of whether to make allowance for the variability in complement strength by varying the amount of amboceptor or by varying the amount of complement (or even by varying the amount of cells, as is done in the Hecht and Weinberg modification of the Wassermann reaction) is a question which has not received adequate consideration, but which seems to have been determined arbitrarily by different workers. A large number of workers (such as Thomsen, Boas, MacIntosh and Fildes, Sormani, Thomas and Ivy, Walker and Swift) use fixed amounts of amboceptor and vary the amount of complement according to its strength. Others (such as Bruck, Citron, Stern, Sachs [Schlossberger]) use fixed complement and varying amboceptor.

There is something to be said for each procedure (varying amboceptor or varying complement). If the practice of keeping a fixed dose of complement and varying the amount of amboceptor gave equally constant results, it would seem to have one considerable practical advantage, namely, that of saving time in the course of a day's work. For since the complement must be in all the tests from the beginning, whereas the amboceptor goes in only after the preliminary incubation, it is possible to set up tests as soon as the complement is prepared, and then while the preliminary incubation is going on, to determine at leisure the proper dose of amboceptor to be used with the particular complement of that day. On the other hand, when the dose of complement is redetermined for each set of tests, the actual setting up of the test must wait until the complement has been titrated.

But while one can undoubtedly produce the same total hemolytic effect by increasing the amount of amboceptor as by increasing the amount of complement, there is grave doubt as to whether the effect in complement fixation is the same. If one attempts to adjust the total hemolytic power by varying the amboceptor instead of the complement, one does not keep constant the actual amount of complement which enters into fixation in the primary incubation, but only the total hemolytic effect of the second incubation. Since the second incubation is merely a test to see how much complement has been absorbed during the first incubation, one deceives oneself if one hopes by varying the amboceptor to keep the complement in the test proper (that is, the first incubation) at a constant level.

For example, if a given positive serum, with a given antigen, contained exactly sufficient of the reacting bodies to bind all the complement present (a quantity of complement which we will represent by



the letter  $x$ ), and we were to repeat the test with the same antigen and serum, but with an equal dose of a complement of twice the activity (namely,  $2x$ ), then only half of the second complement would be bound; and although according to the titration one would have diminished the amount of amboceptor so as to get the same hemolytic effect with the double-strength complement, nevertheless the antigen positive serum complex would not have been able to bind any more of the complement and there would be incomplete fixation instead of complete fixation as in the first instance.

I have proved this repeatedly<sup>5</sup> by showing that when one carries out complement fixation with a specimen of complement exceeding the average strength, it is possible to detect far greater dilutions of positive serum by using a double laking dose of the complement (as determined with two units of amboceptor measured by previous titration with average complements) than by using a double laking dose of amboceptor (redetermining the laking dose of course with the fixed dose of complement used in the test). In other words, if one attempts to make allowance for a strong complement by using a diminished amboceptor instead of a diminished complement dose, one invariably misses certain weak positive tests. Kromayer and Trinchese<sup>6</sup> have shown the same thing by what might be called a *reductio ad absurdum*; they demonstrated that any positive serum will give a negative reaction if a large enough dose of complement be used, but with ordinary doses of complement (after fixation has once occurred) one cannot produce hemolysis (a negative reaction) by the addition of even enormous doses of amboceptor.

It can readily be seen, therefore, that although superficially these two methods of titrating appear to give rather similar results, nevertheless the choice of one or the other indifferently by various workers has probably been the cause of not a few of the discrepancies in results obtained with identical serums.

For the reasons given, then, I am in favor of the practice of titrating complement with a fixed dose of amboceptor and of varying the amount of complement according to its potency. The exact mode of doing this will be discussed below when the subject of dosage of complement is reached. Many workers (including myself) make a practice of doing a preliminary approximate titration of each guinea-pig's serum, and rejecting any which are decidedly below standard. At the same time the control tube is set up for detecting any guinea-pig's serum which contains an excess of natural antisheep hemolysin. This

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5. Ottenberg: Correct and Incorrect Methods of Performing the "Daily Titrations" for the Wassermann Reaction and Other Forms of Complement Fixation, *Jour. Immunol.*, to be published.

6. Kromayer and Trinchese: *Med. Klin.*, 1912, viii, 404.

tube contains merely cell suspension and 0.1 c.c. of complement. It should show practically no laking.

VARIATIONS IN THE "FIXABILITY" OF GUINEA-PIGS'  
COMPLEMENT

In the discussion thus far it has been taken for granted that the complements of different guinea-pigs are equally amenable to fixation. But, as Browning and McKenzie have shown, this is by no means the case. They found that if several complements of equal hemolytic power were tested under the same conditions, sometimes one and one-half times as much of one would be fixed as of another. And repeating the proof of this fact in a different way, I have repeatedly found that of four or five complements of equal hemolytic titer tested simultaneously with alcoholic antigen extracts, the one would give complete inhibition with the positive serum in a dilution of only one-fourth, while another would give equal results with the positive serum in a dilution of one-eighth, and still others with the serum in dilutions of one-twelfth or one-sixteenth.

There is no important fact in the whole subject of complement fixation that has been so neglected as this mysterious phenomenon; indeed most workers seem completely to ignore its existence. And yet probably it is at the bottom of many contradictory reports from different laboratories. It is true that by using the mixed complements of a large number of guinea-pigs one may strike a reasonable average of fixability. And this is one of the unrecognized reasons why reports from large laboratories where many tests are made are more reliable than from small laboratories where few are made, even though the workers be equally competent. But even the average of several specimens is bound to vary unless the number which goes to make the average is impracticably large.

In the future, if the Wassermann reaction is to become standardized, as for instance the production of diphtheria antitoxin has become standardized (and the former task should be no more difficult than the latter), then, among other things, some simple method of testing the fixability of guinea-pigs' complement in the preliminary titration will have to be introduced so that specimens of inferior fixability may be rejected. As the result of experiments now under way I hope in the near future to be able to suggest a method. At present the only way to avoid this source of error is to use mixed complements of a large number of guinea-pigs; four or five should always be used and if possible more.

Before discussing the dosage of complement for the reaction it will be necessary to take up certain considerations connected with the preparation of the sheep cell emulsion and amboceptor, the mode of incubation, and the anticomplementary effects of normal serum and of antigen.



## SHEEP CELL SUSPENSION

The preparation of the sheep cell suspension has undergone but one modification since Wassermann's work and that is a modification which a great majority of workers have adopted. However, there are some workers, as for instance, Citron, who still adhere to the original method.

Wassermann used 1 c.c. of a suspension, which represented five volumes of the whole blood of the sheep diluted with ninety-five volumes of saline (the cells of course having in the meanwhile been carefully washed at least three times by centrifugalization). On account of the fact that various sheep do not agree in the number of red cells per cubic centimeter of their blood, most subsequent workers have used an emulsion representing five parts of compact sedimented washed red blood cells added to ninety-five parts of saline.

This really represents approximately twice as strong a red cell suspension as the original, a fact which does not seem to have received much attention. However, nearly all workers now use 5 per cent. of the sediment as their standard, and it would seem especially important to adhere to this method in case the same sheep is bled repeatedly and hence gradually becomes anemic. In measuring the sediment it is far preferable to measure in a graduated centrifuge tube, reading the upper level of the cell sediment at the end of a definite length of time (ten minutes) in a centrifuge of a definite number of revolutions per minute (from 4,000 to 6,000), rather than to depend on measuring the compact sediment with a graduated pipet.

When defibrinated blood is used (as it is by nearly all workers) it is important that the cell suspension contain no small clots. These contain a great number of red cells and by absorbing an undue share of amboceptor interfere with hemolysis in the particular tubes that they finally reach. They are removed by filtering the defibrinated blood through gauze or by very careful decantation.

The sheep red cells, as well as the complement, represent a variable factor, since it has been shown (Ottenberg and Hopkins<sup>7</sup>) that individual sheep vary considerably in their resistance to hemolysis. This factor is very hard to make allowance for, and probably the best method is to keep one or two sheep in the laboratory and use the blood of the same animal during as long a period of time as possible. However, the range of variation is not nearly so great as that of complement and probably plays a far smaller rôle in the irregularities in the results of the reaction.

## THE TECHNIC OF AMBOCEPTOR TITRATIONS

The absolute titer of the particular amboceptor used is practically a matter of indifference (the term amboceptor will be used here for immune sheep red cell hemolytic serum). It can also be accepted as a fact that after the first few days the potency of amboceptor, if properly preserved, remains practically constant over long periods of time.

In determining the strength of a new amboceptor there are a number of apparently trivial points of technic which, however, may make a considerable difference in the result. The first of these is the element of time. Hemolysis is a reaction which progresses gradually and which is not completed at any one definite time. Therefore, the period of time taken at the end of which the degree of hemolysis is read is to a certain extent arbitrary. Wassermann made the readings in his titrations at the end of one hour in the air thermostat because at the end of that time hemolysis is almost complete. Thomsen, Boas, and others, however, because hemolysis is not absolutely complete at one hour, use a two-hour period. Stern used one and a half hours. Other workers in order to save time do their readings after a half hour (Sormani) or fifteen minutes (Field, Kaliski, and others).

7. Ottenberg and Hopkins: *Proc. Soc. Exper. Biol. and Med.*, June, 1914.



In practice it makes very little difference which particular period is adhered to so long as the same period is always adhered to and so long as the same period is adhered to both in titrations and in the actual performance of the tests. But for purposes of standardizing the reaction it would seem highly desirable that workers agree on some uniform period. The original one-hour period of Wassermann seems rather long on account of the delay in practical work and in view of the fact that over 90 per cent. of hemolysis takes place within the first half hour. The fifteen-minute period is probably too short to allow for slight differences in the speed with which the mixtures assume the temperature of the thermostat due to variations in the thickness of glassware, etc.

Most authors fail to state whether their incubations are in air thermostat or in water bath, yet this is a very essential point, because, owing to the greater speed with which the contents of the tubes become warmed in the water bath, thirty minutes in the water gives about the same result as from forty-five to sixty minutes in the air thermostat. The water bath is preferable because the temperature of the air incubator changes every time its door is opened.

When a very long period is taken, such as two or more hours, another factor plays a considerable rôle, namely, the dissociation of amboceptor. Muir<sup>8</sup> has shown that a certain proportion of amboceptor can be dissociated from the substance of cells on which it has already acted and can then lake additional cells. For these reasons I prefer the half-hour period of incubation in the water bath, which is practically equivalent in result to one hour in the air thermostat. It is greatly to be desired that on this and other similar and more or less arbitrary points of technic some official standards be set by some of the representative organizations of serologists.

Next to the time factor, the manner in which the ingredients are mixed in the titration plays a considerable rôle. This is particularly true if a short time incubation is used. If cells are mixed with amboceptor for ten or fifteen minutes before the complement is added, they absorb the amboceptor and are sensitized and after the addition of complement hemolysis takes place much more rapidly than if the three ingredients are added simultaneously. The advantage of the longer periods of incubation is that (due possibly to dissociation) irregularities due to such factors as these are more or less equalized. Which of the two methods is used (preliminary sensitization of the cells or immediate incubation after mixing all three ingredients) is probably not of great moment. But it is important that the same method should be uniformly used throughout by each worker, a thing which does not seem by any means to be the case, as there are some workers who after titrating by one method use the other method in the actual tests.

A necessary detail which hardly needs to be mentioned to experienced workers, but which, nevertheless, is sometimes neglected, is immediate mixing of the reagents; for instance, if cells are added little by little to amboceptor the result is less laking than if all the cells are added at once, probably because, due to the great avidity of cells for amboceptor, the first cells added absorb more than their share of amboceptor. Likewise if a mixture is made and not shaken at once an uneven sensitization and imperfect laking occurs.

A factor of importance, of course, in determining the unit of amboceptor is the amount of complement used in the determination. Wassermann and the majority of workers following him use 0.1 c.c. of the mixed complement of several guinea-pigs. Some workers, as, for instance, MacIntosh and Fildes,

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8. Muir: *Studies in Immunity*, 1909.

Walker, and Kolmer, use 0.05 c.c. Oluf Thomsen uses 0.08 c.c. of complement, explaining that it is an excess. While 0.1 c.c. of complement does not represent the largest possible excess of complement, nevertheless for the sake of uniformity it had probably better be adhered to. If the amboceptor unit as determined with a very large excess of complement were an absolutely constant and standard thing, it would probably be better to use such a Bordet unit. But (as was found in investigations of the board of health committee referred to above) this is not the case, and even with a great excess of complement, on different days, when the complement is obtained from different guinea-pigs, the amboceptor unit shows slight variation. Furthermore, when one uses amounts of complement decidedly above 0.1 c.c. the natural antishoop hemolysin of guinea-pigs often begins to interfere with the titration. It therefore seems best to titrate the minimal hemolytic dose of amboceptor with 0.1 c.c. of complement on a number of successive days and to take as the amboceptor unit the average of the minimal hemolytic amounts. If this is done, it is probable that workers in different laboratories can at least work with fairly uniform amounts of amboceptor and red cells.

#### THE DOSE OF COMPLEMENT

The factor which more than any other determines the accuracy of the reaction is the dose of complement used, and yet this is a factor on which different workers vary from each other perhaps more than in any other. Wassermann, followed by Citron<sup>9</sup> and a good many other workers, used a fixed dose of complement, namely, 0.1 c.c., the amboceptor and the antigen dose being adjusted so that this amount of complement would represent a sufficient excess to prevent any non-specific fixations. Subsequent workers have modified the amount or the principle of determining the amount of complement with the general idea that the smallest amount compatible with safety should be used.

In reasoning on the subject certain factors which interfere with the hemolytic action of complement must be recognized. Practically all the substances used as antigens, if taken in a large enough dose, interfere with the action of complement. Likewise, human serum, whether normal or syphilitic, interferes to some degree with the action of complement, and this anticomplementary power of serum is very variable for different individuals, and furthermore, it depends on the age and condition of the serum. Beside this it is possible that some normal serums have a very small but appreciable power of reacting with antigen in a nonspecific way. It was to prevent false positive results due to these factors that Wassermann established his standard of a hemolytic system of double strength (two units of amboceptor) and the dose of antigen the double of which should not of itself at all inhibit the action of the hemolytic system used.

It has appeared to most of the subsequent workers that this system provides a somewhat larger safety zone than is needed, and the general

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9. Citron: Immunity, translated by A. L. Garbat, 1914.



effort has been to find some method of making the reaction more delicate by diminishing the amount of complement, while at the same time making sufficient allowance for the nonspecific anticomplementary factors mentioned. As a matter of fact, the great majority of workers now use an amount of complement which is considerably less, generally from one half to three fifths of the amount prescribed by Wassermann.

Wassermann's quantity, 0.1 c.c., is an actual excess and really represents not merely two units of complement with two units of amboceptor, but probably three or more units of complement with two units of amboceptor. This can readily be shown by retitrating the amboceptor unit which has already been determined with 0.1 c.c. of complement, but doing the titration with smaller amounts of complement. It is found that a considerably smaller amount of complement, for instance sometimes 0.06 c.c., will give the same amboceptor unit. Kromayer and Trinchese<sup>10</sup> pointed this out in 1912. They showed that if an arbitrary (not minimal) amount of complement is used with descending amounts of amboceptor, the least dose of amboceptor that gives complete laking will be found to do so with a still smaller dose (up to 30 per cent. less) of complement. This was confirmed by M. Stern<sup>11</sup> in 1914.

I have often repeated the experiment in the form given below. The amboceptor unit was first determined with 0.1 c.c. complement as follows:

I. Complement 1 to 10, 1 c.c.

Amboceptor (No. 267, 1 to 200).....	0.15	0.2	0.24	0.28	0.0
Thirty-minute reading .....	c? <sup>*</sup>	c	c	c	

\* The letter "c" signifies complete laking.

The amboceptor unit was thus found to be between 0.15 c.c. and 0.2 c.c. of the 1 to 200 dilution and was estimated as being 0.18 c.c. Now a titration of the identical complement with two of these amboceptor units, namely 0.36 c.c. was done as follows:

II. Amboceptor 0.36 c.c. (2 units)

Complement 1 to 10.....	0.25	0.3	0.35
Thirty-minute reading .....		c	c

It was thus seen that with two amboceptor units the least amount of complement which would give complete laking was 0.3 c.c. of the 1 to 10 dilution. This might therefore be termed the complement unit with two units of amboceptor.

Now with two such units of complement, namely 0.6 of the 1 to 10 dilution, the amboceptor was again titrated and on comparing this with the first of the three titrations it is seen that the amboceptor unit obtained with 0.6 c.c. of complement is practically identical with the amboceptor unit obtained with 1 c.c. of the 1 to 10 complement.

III. Complement 1 to 10, 0.6 c.c. (2 units)

Amboceptor (No. 267, 1 to 200)....	0.15	0.2	0.24	0.28
Thirty-minute reading .....	c?	c	c	c

10. Kromayer and Trinchese: *Med. Klin.*, 1912, No. 10, p. 404, No. 41, p. 1670.

11. Stern, M.: *Ztschr. f. Immunitätsforsch. u. exper. Therap.*, 1914, **22**, 130.



Thus, if Wassermann tests were set up with 0.6 c.c. of the 1 to 10 dilution of this complement and two units (0.36 c.c. of the 1 to 200 dilution) of this particular amboceptor, the amount of complement would really represent two units of complement as determined with two units of amboceptor; and it is seen that 1 c.c. of 1 to 10 complement (namely, 0.1 c.c. complement) really represents three or more units. The author has found in practice, as have many other workers, that the smaller of these two amounts, namely, two units of complement and amboceptor, represents (with simple alcoholic extracts as antigens) an amply sufficient margin of safety, and gives far more delicate results than the larger, arbitrary dose of complement. The reason that led the majority of workers to use smaller amounts of complement was not, of course, any theoretical consideration such as these, but the practical experience that smaller doses were safe and gave better results. The question, however, by what criterion to decide just what dose to use has not been settled.

It is obvious that it would not be safe to use one unit of amboceptor with one unit of complement for the reason that this would make no allowance for the anticomplementary effects of antigen and of patients' serum. What is needed, of course, is one complete unit of complement (that is sufficient to produce complete laking) above and beyond that amount of complement which is neutralized by the nonspecific complement-binding powers of the anticomplementary agents present in the reaction.

Maslakowitz and Lieberman,<sup>12</sup> Browning and McKenzie<sup>13</sup> in 1909, and Thomsen<sup>14</sup> in 1910 made the first steps in this direction. Thomsen proposed to determine the free unit of complement by titrating the complement in the presence of antigen, and using in the tests that amount of complement which (with two units of amboceptor) just gives complete laking in the presence of the arbitrary amount of antigen used. In the serum control tube he used one unit (as determined with two units of amboceptor, but of course not in the presence of antigen). This method is correct in principle, but it does not go far enough, since it disregards the slight, but frequently present, anticomplementary effect of the patient's serum, with the result (as the author can testify from having tried the method extensively) that the patient's serum is occasionally found anticomplementary in the serum control tube, so that one cannot with safety report the result of the test.

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12. Maslakowitz and Lieberman: *Ztschr. f. Immunitätsforsch. u. exper. Therap.*, 1913, p. 804.

13. Browning and McKenzie: *Ztschr. f. Immunitätsforsch. u. exper. Therap.*, 1909, **2**, 459.

14. Thomsen, Oluf: *Ztschr. f. Immunitätsforsch. u. exper. Therap.*, 1910, **7**, 389.

Margarete Stern<sup>11</sup> in 1914 apparently tried to get around this difficulty by increasing the hemolytic strength of the serum control tube, but not of the test proper. She kept the dose of complement constant (0.1 c.c.) and titrated the amboceptor in the presence of the antigen, using in the test that amount of amboceptor which caused complete laking in the presence of the antigen; in other words, using what in effect is equivalent to one free unit of amboceptor above the amount neutralized by the anticomplementary effect of the antigen. In the serum control tube she used not one but one and a half units of amboceptor. This seems a dangerous and deceptive practice, since the extra allowance of amboceptor for the anticomplementary effect of patient's serum is omitted precisely in that tube in which it is most important, the test itself.

The most ingenious and complete method of making accurate allowance for the anticomplementary factors in the test is the method of Browning and McKenzie,<sup>18</sup> 1909. These authors did a daily determination of the complement unit, using in this determination five units of amboceptor. They then set up a series of serum tubes containing one, two, three and four complement units for each serum. For the antigen control they likewise set up, with their arbitrary dose of cholesterinated beef heart antigen, tubes containing one, two, three and four units of complement. For the test itself they used a series of tubes containing 5, 7, 10, 15, 20, 30 and 40 units of complement. They thus determined simultaneously the anticomplementary value as measured in units of complement of the antigen and of each serum tested. They require in order that a serum be regarded as positive that it give inhibition with at least five complement units over and above the number of complement units inhibited by the serum itself and by the antigen. This system surmounts many of the obstacles which are encountered, but the use of the enormous amounts of complement in the last two or three tubes of the test (namely, twenty, thirty and forty units) is so wasteful as to seem impracticable, and as a matter of fact is seldom necessary.

Indeed, the present author's trial of this method demonstrated that in order to detect many positive serums which were detected by other methods (Thomas and Ivy, Citron, Walker and Swift) it was necessary to set up tests not with seven, ten, fifteen, etc., units of complements, but with three, five, seven, etc., units (titrated, of course, after the directions of Browning and McKenzie). In other words, Browning and McKenzie's stipulation that in order to be regarded as positive a serum must fix five or more units is found to be far too exacting. The fixation of two or more units of complement can (with cholesterinated beef heart extracts) be safely regarded as specific.

With the slight changes suggested the method of Browning and McKenzie deserves very careful trial. One of its great advantages is that with it serums which are in themselves to a considerable degree anticomplementary can be tested without difficulty, as the serum control tubes represent a titration of this anticomplementary property and one



simply has to subtract the number of complement units found inhibited in the serum control in order to make allowance for the anticomplementary property of the serum.

One of the most satisfactory methods of determining the complement doses according to the principles laid down by Maslakowitz and Lieberman<sup>12</sup> and by Thomsen,<sup>14</sup> is the method proposed by Thomas and Ivy\* in 1914. It follows the method of Thomsen fairly closely, but it has the advantage of making an allowance for the anticomplementary effect of normal serum, as is done in Browning and McKenzie's method, and it might be called a combination of Thomsen's and Browning and McKenzie's methods.

It consists of using in the test the minimal amount of complement which gives complete laking when titrated in the presence of the arbitrary dose of antigen and the amount of negative serum used in the test. The negative serum for this titration is pooled negative serum from several patients. Before the amboceptor and cells are added for the titration, the complement, serum and antigen are incubated as for the test proper, so that the anticomplementary effects of antigen and of serum (whether normal or syphilitic) are imitated under exactly the same conditions as those under which they exert their influence in the test. The dose of amboceptor used represents approximately two or three units. It is necessary to pick out such negative serums as contain no natural antisheep amboceptor, otherwise the complement titration is illusory.<sup>15</sup> In the serum control tube the amount of complement used is that which gives complete laking in an exactly similar titration done in the presence of pooled negative serum, but not of antigen. The amount of complement in each tube, therefore, represents exactly one free unit above the amount neutralized by the anticomplementary properties of pooled negative serum in one case, and of negative serum and antigen in the other.

I have used this system extensively and believe that it represents the most delicate and accurate way of determining the safe minimal dose of complement to use. In practice, however, it was found not safe to use the absolutely smallest amount of complement which just gave complete laking, for the reason that human serums, even when fairly fresh and recently inactivated, vary slightly in their anticomplementary property. Hence, if one uses the minimal amount which in the titration gives complete laking, then a serum which has slightly more anticomplementary property than the pooled negative serum used in the titration will give an unsatisfactory result, due to the serum control tube being incompletely laked.

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\* Thomas, B. R., and Ivy, R. H.: *Applied Immunology*, 1915.

15. Ottenberg and Frazier: *Jour. Infect. Dis.*, 1915, xvi, 119.



For example, if the three normal serums entering into the pool have anticomplementary values which we will represent by the figures, 5, 3, and 1, then the anticomplementary value of the pool would be 3 (the average) and the test done with the serum whose anticomplementary value was 5 will not lase completely in the serum control tube or the main tube. For this reason the author makes a practice of using the next dose above that which gives complete laking both in the titration with pooled negative serum and in that with pooled negative serum plus antigen. As the tubes in the titration differ by only 0.005 c.c. of complement, this represents only a very minute amount above the one free unit.

It is generally agreed that with cholesterinated antigen a stronger hemolytic system is required than with simple alcoholic extracts, but there is the same lack of uniformity as to the method of determining the strength of this hemolytic system as there is among those who work with simple alcoholic antigens. Thus Sachs,<sup>16</sup> according to Schlossberger, uses the fixed dose, 0.1 c.c. of guinea-pig complement, but uses four units of amboceptor, and, as a further precaution, uses half the amount of patient's serum employed in the original Wassermann reaction. Field<sup>17</sup> specifies from six to eight units of amboceptor with from three to four units of complement, although he does not describe exactly how these units are determined. Walker<sup>18</sup> and Swift use two units of amboceptor and two units of complement, the unit of complement being determined with a single unit of amboceptor, so that the amount of complement is generally about 0.1 c.c., and the actual strength of the hemolytic system is approximately fourfold. The work of Browning and McKenzie described above shows that the same principle of subtracting the anticomplementary effect of antigen and of patient's serum from the total amount of complement fixed, as is used in Thomsen's and in Thomas and Ivy's methods, with alcoholic antigen can be applied to the cholesterinated antigen. But the amount of free complement which they specify must be fixed (five units) over and above these anticomplementary effects in order that the reaction may be regarded as a positive one is of course greater than with the alcoholic extracts, and, according to my experience, as mentioned above, is too great.

#### PATIENT'S SERUM

The one element in the reaction the handling of which has undergone the least modification is the patient's serum. The amount prescribed by Wassermann, 0.2 c.c., as being sufficient to give positive results and yet safe within the range of the amount which may give a nonspecific result has been adhered to by the great majority of workers.

16. Sachs: *Ztschr. f. Immunitätsforsch. u. exper. Therap.*, 1913, **20**, 115.

17. Field: *THE ARCHIVES INT. MED.*, 1914, **13**, 790; *Jour. Am. Med. Assn.*, 1914, **62**, 1620.

18. Walker, I. C.: *THE ARCHIVES INT. MED.*, 1914, **11**, 563.

Sachs, according to Schlossberger (1913), Browning and MacKenzie (1909), Thomas and Ivy (1914) and some others used a half of the usual dose, namely, 0.1 c.c. Schlossberger acknowledges that the larger dose increases the sensitiveness of the reaction and is safe provided one uses a slightly more active hemolytic system. It is interesting to note that all the workers who use the smaller dose of patient's serum use relatively very large doses of antigen extract.

On the other hand, there have been a number of attempts, such as those of Kromayer and Trinchese<sup>9</sup> (1912), Ledermann<sup>10</sup> (1913) and of Thiele and Embleton,<sup>20</sup> to increase the delicacy of the reaction by increasing the proportion of patient's serum. Thiele and Embleton, who use up to five times the usual quantity, attempt to compensate for this by using a relatively much smaller amount of antigen than other workers, and although the general opinion of other workers seems to be that this method is unsafe, it does not appear to have been tried by others as it deserves to be. Field, in addition to using the regular amount, uses a tube containing double the amount of serum, but he regards a positive result obtained with this amount in the presence of a negative result with the regular amount as being merely a "suspicious" reaction.

With spinal fluids the use of far greater amounts has been found to be safe and to detect reactions which would otherwise be lost. The method introduced by Hauptmann\* of using graded amounts up to 1 c.c. is now generally followed. Some workers even carry the dosage up to 2 c.c., and as long as the control is satisfactory this seems justifiable.

#### INACTIVATION

Practically all workers inactivate the patient's serum by heating in a water bath for a half hour at 56 C. This is not done, however, with spinal fluids, unless they are several days old before being tested. Inactivation of course has for its purpose not only the removal of the unknown additional amount of complement in the patient's serum, but the destruction of the anticomplementary effect of some serums, the latter being the more important.

The observation of Seligman and Pincus<sup>21</sup> that the use of active patient's serum leads to a certain proportion of false positive reactions has received general acceptance. Only Graetz<sup>22</sup> (1913) denied this and believed that with proper technic it was possible to get perfectly reliable results with active serum. Noguchi showed that the false positive results with active serum were due to certain impurities, probably lipoid-protein combinations in the antigen, which were removed by his method of acetone precipitation of the alcoholic extracts, and he recommended that with his acetone insoluble lipoids active serum be used. In spite of this and of the undoubted fact that heating destroys some of the positive reacting substances, practically all serologists at present agree that inactivation is necessary to avoid nonspecific results.

#### THE SERUM CONTROL TUBE

The amount of patient's serum to be used in the serum control tube without antigen has undergone but one modification, and this is one which is very widely used.

\* Hauptmann and Hössli: München. med. Wchnschr., 1910, **57**, 1581. Hauptmann: Deutsch. Ztschr. f. Nervenhe., 1911, **42**, 240.

19. Ledermann: Med. Klin., 1913, No. 50, p. 2071.

20. Thiele and Embleton: Ztschr. f. Immunitätsforsch. u. exper. Therap., **16**, No. 4, p. 430.

21. Seligman and Pincus: Ztschr. f. Immunitätsforsch. u. exper. Therap., 1910, **5**, 377.

22. Graetz: Med. Klin., 1913, **9**, No. 45, p. 1858; *ibid.*, No. 46, p. 1898.



Wassermann himself prescribed that a control tube containing double the amount of patient's serum used in the main tube should not give inhibition, but experience has shown that he was more cautious than was necessary, and a great many workers now use the same amount of serum in the serum control as in the main tube. Some, such as Sachs (according to Schlossberger), Walker and Swift, and Bruck,<sup>23</sup> adhere to the double dose.

The use of the double dose of serum in the control tube superficially appears to be an additional factor of safety. In reality this is not the case. It is true that if the patient's serum possesses a certain amount of anticomplementary property, such as might lead to a false positive result, this is accentuated and hence more easily detected if the double dose is used. On the other hand, if the patient's serum happens to contain a considerable amount of antish sheep hemolysin, the control will contain double as much as the main tube, and the more complete and rapid laking in the control tube can falsely give the appearance of a positive result. The identical dose of serum in the control as in the main tube certainly gives a more accurate picture of the effect the serum is having in the main tube, and this is the real object of a control tube. For these reasons I believe that the dose of serum in the control tube should be the same as that in the main tube.

#### THE DOSAGE OF COMPLEMENT IN THE SERUM CONTROL TUBE

To a worker who is accustomed to following the original Wassermann technic rigorously the use of different doses of complement in the serum control tube and in the main tube, as in the methods of Thomsen, of Boas, and of Thomas and Ivy, or of a different dose of amboceptor in the two tubes, as in the method of Stern, at first seems to deviate from the fundamental principles of the test. But, as a matter of fact, it does not, since it merely makes allowance for the different anticomplementary factors present in the two tubes. In fact, this method really offers a safer result than the original one of equal doses of hemolytic components in the control and main tubes. For in the original method in order to be sure to have enough complement in the main tube to overcome any anticomplementary property of the antigen, the dose of complement in the control tube, being the same as in the main tube, is considerably in excess. But this excess of complement in the control tube is enough to mask a certain degree of anticomplementary effect of the serum; and false positive results due to the addition of anticomplementary effects in the main tube are therefore harder to detect.

Thus, suppose (an actual instance) the titration shows that for the

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23. Bruck: Serodiagnosis of Syphilis, 1909.



original Wassermann system the required dose of complement (two units) is 0.1 c.c., and the amount needed for a system such as Thomas and Ivy's in the tube containing both antigen and serum is 0.09 c.c., and in the control tube with serum alone 0.06 c.c. In the original system, of course, the control tube with serum alone gets the same dose of complement as the main tube, 0.1 c.c. But this excess may be enough to mask the anticomplementary effect of some particular serum and nevertheless this anticomplementary effect, added to the slight anticomplementary effect of the antigen in the main tube, may be enough to give falsely a weak positive result.

To make this clearer, consider the above instance; the unit of complement was found to be 0.05 c.c., the unit of complement as determined in the presence of pooled negative serum 0.06 c.c., the unit as determined in the presence of serum and antigen 0.09 c.c. From these figures it is clear that the pooled negative serum has an anticomplementary value of 0.01 c.c., and the antigen an anticomplementary value of 0.03 c.c. Now it is clear from this that in the original Wassermann, in which the identical amount of complement, 0.1 c.c., is used in both main and control tubes, in the average case in the main tube there is 0.06 c.c. free complement, and in the control tube 0.09 c.c. But suppose that among the serums to be examined on a particular day there was one whose anticomplementary value was as high as 0.05 c.c. of complement, then in the original Wassermann this would still leave in the control tube 0.5 c.c. of complement, sufficient to give complement laking in the control tube, while in the main tube this anticomplementary effect added to the slight (0.03 c.c.) anticomplementary effect of the antigen would use up 0.08 c.c. of complement and leave only 0.02 c.c., in other words, would give the appearance of partial inhibition or a false positive reaction, since the control tube would be completely laked. On the other hand, if the tests were set up so that an exact unit above the average anticomplementary effect of human serum was used in the control tube, rather than the huge excess of the original Wassermann, the anticomplementary property of this serum would be detected readily and a false positive result would not be reported.

This is undoubtedly one of the reasons why all workers refuse to attribute much diagnostic value to weak inhibitions with the original Wassermann system. It is likely that the practice of more accurate adjustment of the complement dose in the serum control tube will make weak inhibitions more significant. It is the particular advantage of the system of Browning and McKenzie that it makes accurate allowance for the anticomplementary effect of each serum tested.

In the original technic of Wassermann the attempt was made to avoid this source of error by the use of the double quantity of patient's serum in the serum control tube. And for those who adhere to the original method in

this regard, using the same strength of hemolytic system in the serum control tube as in the main tube, this is probably the safer procedure. Unfortunately, however, in case the patient's serum contains any natural antisheep amboceptor, the control tube will contain twice as much as the main tube; and this, if one depended on the natural antisheep amboceptor, could easily give one a false weak positive result. Thus, for instance, if the ordinary dose of serum (0.2 c.c.) contained not quite enough antisheep amboceptor to produce complete laking, the control tube containing twice as large a dose of serum would contain more than enough to produce laking and one would get the appearance of partial inhibition in the main tube with complete laking in the control tube. Therefore, if the test is done with double serum control, one must invariably add amboceptor and disregard any appearance of a positive reaction obtained with natural amboceptor alone. To do this, however, as will be shown below, causes one of necessity to miss a certain small proportion of weakly positive reactions, which become negative due to the excess of natural amboceptor present. It is possible, of course, to avoid missing these by repeating all the tests that contain much natural amboceptor after its absorption or by subjecting all serums to absorption in the first place. This subject will be further discussed below under the heading of natural antisheep amboceptor.

#### THE MANAGEMENT OF NATURAL ANTISHEEP HEMOLYSIN IN THE PATIENT'S SERUM

Many authors have referred to the frequent occurrence of natural antisheep amboceptor in appreciable amounts in human serum.<sup>24</sup> The well-known modification of Bauer, as well as that of Hecht and Weinberg, take advantage of this; and the substitutes for the Wassermann method, such as that of Noguchi, are devised chiefly to eliminate the error caused by it. The occasional occurrence of a great excess of such natural amboceptor is also well recognized. Thus Ottenberg and Frazier found in 2,158 tests that 955 serums (44 per cent.) had an appreciable amount of natural amboceptor, and that 476 (21 per cent.) had sufficient to produce complete laking without further addition of amboceptor.

In obtaining these figures the complement was always titrated and the amount used represented two complement units, as discussed above under the head, dose of complement, and was generally about 0.06 or 0.08 c.c. Those samples of complement which were below the average were rejected and not used, while those which were above the average in activity were used in a smaller dose so as to equal the average. If all the human serums had been tested with the arbitrary amount (0.1 c.c.) of guinea-pig complement used in the original Wassermann reaction, it would have been found that a far larger proportion show natural amboceptor, as smaller amounts of natural amboceptor are brought out by greater amounts of complement. (In fact with larger amounts of complement over 90 per cent. of human serums are found to contain some antisheep amboceptor.) Thus when Wassermann tests are done using a fixed amount of guinea-pig serum as complement, as is done by

24. Wassermann, Neisser, Bruck and Schucht: *Ztschr. f. Hyg. u. Infektionskrankh.*, 1906, **55**, 451. Jacobaeus: *Ztschr. f. Immunitätsforsch. u. exper. Therap.*, 1911, **10**, 321. Rossi: *Ztschr. f. Immunitätsforsch. u. exper. Therap.*, 1911, **8**, 615. Dexter and Cummer: *THE ARCHIVES INT. MED.*, 1912, **9**, 605. Olmstead: *Med. Rec.*, New York, 1914, **85**, 341.



Citron, Stern, Sachs, and many others, the possibilities of error due to the presence of natural amboceptor are really much greater than when the amount of complement is varied according to its strength.

Although some workers pay no attention to natural amboceptor, a perusal of the literature and my own experience leave no doubt that if the presence of natural amboceptor is disregarded a certain small percentage of false negative reactions is obtained. Some authors have undoubtedly exaggerated the proportion of such cases. Nevertheless, it is obvious that the occasional coincidence of a large excess of natural amboceptor with a small amount of so-called syphilitic antibody will inevitably lead to this error. In spite of the many methods which have been proposed to obviate this difficulty there is none entirely satisfactory. The absorption methods are accurate but cumbersome, and sometimes render serums anticomplementary (Sachs' phenomenon). Some observers make a preliminary test, often called the Bauer control tube, to determine the natural amboceptor, and then add immune amboceptor only to those serums that need it. Others, like Graetz<sup>25</sup> and like Snow and Cooper,<sup>26</sup> make a routine practice of absorbing the natural amboceptor from all serums. Browning and McKenzie<sup>13</sup> use ox cells instead of sheep cells, because natural antioxy hemolysin is of less frequent occurrence in human blood than antioxy hemolysin.

In general, the error due to natural hemolysin is greatest in those systems which use relatively large doses of complement; and in the systems which (like those of Thomsen<sup>14</sup> and Thomas and Ivy\*) reduce the complement to one free unit this error is reduced to a minimum.

The method which I have adopted for use with the system containing two hemolytic units of complement originated with Kaliski and is similar to the Bauer method, but simpler. It consists of the addition of unsensitized sheep cells to all the tubes and incubation for ten minutes in the water bath at 37 C. At the end of this time those tests which contain a considerable excess of natural amboceptor show complete laking in the control tubes, and to these tests no amboceptor is added. To all the other tests the usual amount of two units of amboceptor is then added, and all the tests are incubated for the full period. Thus, the addition of still more amboceptor to those tests that already contain an excess is avoided.

It must be borne in mind that even with this method those rather exceptional serums that have a very great excess of natural amboceptor combined with weak Wassermann reacting bodies may still give false negative results. These serums (recognized by rapid laking in all the tubes) are retested after absorption of the natural amboceptor. How

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25. Graetz: *Med. Klin.*, 1913, **9**, 1858, 1898.

26. Snow and Cooper: *Am. Jour. Med. Sc.*, 1916, **152**, 185.

\* Thomas and Ivy: "Applied Anatomy," 1915.



valuable absorption has proved in these cases may be seen from the fact that in the last eight months of 144 negative reacting cases which were retested after absorption of a considerable excess (two or more units) of natural hemolysin, thirty-six cases became definitely positive. Of these, seventeen became weakly positive (plus or double plus) and nineteen became strongly positive (triple plus or quadruple plus). All of these were cases in which there were good clinical grounds for suspecting syphilis.

If the reliability of the reaction is to be increased it seems to me that all those methods of complement fixation which depend exclusively on the natural hemolysin of human serum, as well as those like the Hecht-Weinberg reaction, which depend also on the natural complement, should be rejected. Ingenious as these methods are, they should be regarded as tours de force which tend away from, rather than toward, accuracy and reliability.

#### PRIMARY INCUBATION

Bordet and Gengou, Wassermann, and other early workers in complement fixation experimented with various periods of time and various temperatures for the incubation during which the fixation of complement occurred. It was found that the temperature does not play a very great rôle, but that fixation occurs somewhat better at temperatures approximating 37.5 C. than at other temperatures. It was found also that fixation is not a reaction which occurs suddenly, but one that occurs progressively and that in general the longer the time the more complete the fixation of complement. However, as it was found that at 37.5 C. complement deteriorated after several hours; and as most of the fixation possible occurred in the first hour, Wassermann established the rule of doing complement fixations at 37.5 C. for one hour, and until recently this was adhered to by most workers.

On account of the fact that fixation goes on almost as well at lower temperatures (from 8 to 10 C.) as at body heat, and that at these temperatures the deterioration of complement in longer periods than one hour is exceedingly slow, Jacobsthal,<sup>27</sup> 1910, advocated doing the preliminary incubation in the icebox at from 8 to 10 C. for longer periods (from three to four hours). This, the so-called icebox method, has been tried out by a great many other workers (Altman,<sup>28</sup> 1913, Leredde and Rubinstein,<sup>29</sup> 1914), and has been found to give results decidedly superior to the one-hour incubation at 37.5 C., and to give no false positive results whatever. Very much longer periods, such as from twelve to eighteen hours in the icebox, have been found unsatisfactory.

In many hundreds of tests done in parallel with icebox and with thermostat (37.5 C.) incubation, I have been able to confirm this, and I

27. Jacobsthal: *Deutsch. Med. Wchnschr.*, 1913, **39**, 1337; *ibid.*, No. 29, p. 1435.

28. Altman: *Arch. f. Dermat. u. Syph.*, 1913, **116**, 871.

29. Leredde and Rubinstein: *Bull. Soc. franç. de dermat. et de syph.*, 1913, No. 2, p. 93.

Year	Author	Cell Emulsion	Amboceptor Dose	Complement Dose	Patient's Serum	Serum Control	Antigen Dose	Antigen Control
1906	Wassermann...	1 c.c., 5% of full blood	2½ units (at 1 hr. with 0.1 complement)	Fixed dose 0.1.....	0.2	0.4	½ amt. not of itself anti-complementary (watery, later alcoholic extract)	Double dose
1909	Bruck.....	1 c.c., 5% of full blood	3-4 units.....	Fixed dose 0.05.....	0.2	0.4	½ amt. not itself anticomplementary (alcoholic extract syphilitic liver)	Double dose
1909	Citron.....	1 c.c., 5% of full blood	2 units (at 1 hr. 0.1 complement)	Fixed dose 0.1.....	0.2 and 0.1	0.2	½ amt. not itself anticomplementary (alcoholic extract normal organs)	Double dose
1913	Sachs (Schlossberger)	1 c.c., 8% of sediment	4 units (unit at 1 hr. 0.1 complement)	Fixed dose 0.1.....	0.1	0.2	½ amt. not itself anticomplementary (cholesterinated beef heart)	Double dose
	Noguchi.....	1 c.c., 5% of sediment	2 units (unit at 1 hr. excess complement)	2 units (unit = smallest amt. that lyses in 2 hr. unit of amboceptor.)	0.2	0.2	½ amt. not itself anticomplementary (acetone insoluble lipoids) (0.2 of 0.3 % emul. of lip.)	Double dose
1914	Thiele and Emerton	0.4 c.c., 5% of sediment	2 units.....	1¼ units (0.2 by calculation, but not stated clearly)	0.2 and 1.0	0.2 and 1.0	About 0.2 c.c. of 1/10 dil. acetone insol. lip. (said to be 50 to 100 units). Acetone insol.	Antigen shows no anticomplement
1914	Walker and Swift	1 c.c., 5% of sediment	2 units (unit at 1 hr. 0.05 complement air incubation)	2 units (generally 0.08-0.1) (unit 1 hr. with 1 unit of amboceptor)	0.2	0.4	Not over ¼ amt. that is completely anticomplementary (cholesterinated human and beefheart)	Single dose
1914	Field.....	1 c.c., 5% of sediment	6-8 units.....	3-4 units .....	0.2	0.2	½ anticomplementary dose (= dose shows beginning anticomplementary effect)	.....
1910	Browning and McKenzie	2 c.c., 5% of ox blood (5% of whole blood)	5 units (unit at 1 hr. with 0.2 complement)	1, 2, 3, 4 units for antigen and serum control (a and b) 7, 10, 15, 20, 30, 40, units for test (c) (unit 1 hr. with 5 amboceptor units, unit = 0.1-0.2)	0.1	0.1	Arbitrary (very large) dose 1.2 c.c. 1/6 dil. cholesterolin beef heart	Single dose
1910	Oluf Thomsen.	5% of sediment	2 units (unit at 2 hr. with 0.08 complement, an excess)	1 unit for control tube 0.04. 1 unit above amt. neutralized by antigen for main tubes (0.08) (unit 2 hr. with 2 units of amboceptor)	0.2, 0.1, 0.05, etc.	0.2	Arbitrary fixed dose (0.2) of alcoholic heart extract	Single dose
1913	Harold Boas...	5% of sediment	2½ units (unit at 2 hr., 0.1 complement)	Same as Thomsen.....	0.2, 0.1, 0.05, etc.	0.2	Like Thomsen .....	Single dose (anticomplement in doubt)
1911	Sormani.....	5% of sediment	8-12 units so that laking is finished in ½ hour	Same as Thomsen except ½ hr. laking and excess of amboceptor	0.2	0.2	Arbitrary dose (0.25-0.3) alcoholic heart extract	Single dose
1914	Margarete Stern	5% of sediment	1½ units in serum control; 1 unit above amt. used up by antigen in main tube (1½ hr. with 0.1 complement)	Fixed dose 0.1.....	0.2	0.2	Arbitrary dose (0.3 alcoholic extract), 5 different antigens for each test	Single dose
1914	Thomas and Ivy	5% of sediment	2-3 units (arbitrary fixed dose — 1 c.c. 1/1000, whose titer is 1/2000 or 1/3000)	1 unit above amt. neutralized in prelim. incubation by antigen and pooled serum (generally about 0.05). For serum control 1 unit above amt. neutralized by serum	0.1	0.1	Arbitrary dose (0.2 alcoholic extract of syphilitic liver)	Single dose



# OF DIFFERENT WORKERS FOR WASSERMANN REACTION \*

Incubation a. Prelim. b. Final	Quantitative Reading	Special Points	Natural Hemolysin	Mode of Titration
1 hr. } 37.5 C. 1 hr. } air (or water bath)	Degree of laking.....			Daily determination of amboceptor unit with 0.1 c.c. complement
1 hr. } 37 C. 1 hr. }	.....	Sensitizes cells 15 minutes...	Acknowledged as unavoidable source of error	Daily amboceptor titration with 0.05 c.c. complement
1 hr. } 37 C. 1 hr. } air	Tube 1 full doses, Tube 2 half doses ant. and serum. 1 and 2 complete +++++, 1 comp. 2 partial +++, 1 comp. 2 none ++, 1 partial 2 none +	Sensitizes cells .....		Daily amboceptor titration with 0.1 c.c. complement
1 1/4 hr. } 37 C. 1-2 hr. } air	Estimated by descending doses of antigen	Sensitized cells. Uses 4 doses antigen; as control uses 4 double doses; reads tube whose double antigen is completely laked		Daily amboceptor titration with 0.1 c.c. complement
1 hr. } 37 C. 2 hr. } water bath read 4-6 hr. after room temp.	One extra tube with half dose serum. Readings after Citron scale (though both tubes get full dose antigen)			(Daily complement titration with one amboceptor unit)?
			Disregarded .....	Daily complement and amboceptor titrations ("simultaneous")
1 hr. } 37 C. 1 hr. } air	Degree of laking.....	Uses always 3 antigens; positive result with any one is conclusive	"Bauer tube" with 1/2 dose serum. If completely laked may absorb	Daily complement titration with one unit amboceptor
	Six arbitrary doses antigen (similar to Sormani)			Daily complement titration (not described exactly)
1 1/4 hr. } 37 C. 1 1/4 hr. }	Read the number of complement units fixed above sum of units fixed by extract alone and serum alone. Positive only if 5 or more units		Ox cells used because hemolysin for them is rarer	Daily titration of complement unit
1 hr. } 37 C. 2 hr. } reads next day	Descending doses of serum (0.2, 0.1, 0.05; if needed, 0.025, 0.012, 0.006). Hemoglobin scale; reports positive only if over 20% inhibition	Anticomplementary serums tested by titrating amt. complement serum alone destroys and repeat test with this amt. added to all tubes		Daily titration of complement unit and of complement unit in presence of antigen dose
3/4 hr. } room 3/4 hr. } 37 C. 2 hr. reads next day	Like Thomsen .....			Like Thomsen
1 hr. } 37 C. 1/2 hr. }	Descending antigen doses (smallest is least that gives + result with secondary syphilitic serum. Five tubes reads, 2/10, 4/10, 6/10, 8/10, 10/10 positive)			Like Thomsen
1 hr. } 37 C. 1 1/2 hr. }	None .....			Daily titration of amboceptor unit (with 0.1 c.c. complement) and of amboceptor unit in presence of dose of antigen
1 hr. } 37 C. 1 hr. }	Increasing units of complement (2, 3, 4, or more). Units determined in separate titration are added to dose used in main tube. Thus 2 units in test means dose in main tube plus 1 bare unit			Daily titration of complement unit (with fixed dose amboceptor) and of complement unit in presence of antigen and normal serum together and of normal serum separately
4 hr. } ice box, 8 C. 1 hr. water bath, 37 C.	Tube 1 full dose, Tube 2 half dose serum, but both full dose antigen; readings on Citron scale	Separate amboceptor titration of each pig; rejects those that are below average. All tests done in duplicate	Receives no attention. Each guinea-pig is, however, tested for natural hemolysin and rejected if any is present	Daily determination of amboceptor unit with 0.1 c.c. complement 1 hr. in water bath 37 C.



believe that at least with the alcoholic antigens the icebox method gives by far the most delicate and accurate results. In experiments with weakly positive serums I have found that at very low temperatures (from 0 to 2 C.) fixation is not so good, but that at the temperature of the ordinary icebox (from 8 to 10 C.) fixation in a given time is almost as good as in the thermostat, and in the longer period (four hours) is much better. With cholesterinated antigens, however, the icebox method gives a considerable proportion of false positive reactions, and with that type of antigen, therefore, it should not be used. Swift, who is one of the principal advocates of cholesterinated antigen, stated recently his belief that with simple alcoholic extracts incubated in the icebox the results were practically identical with those obtained with cholesterinated antigen in the thermostat.

On the other hand, it is claimed by Altman<sup>29</sup> that there are some serums, particularly in primary syphilis, that give a better reaction at 37 C. For this reason, possibly, it is wise to combine the two methods (icebox and thermostat incubation) so that if there is any specific difference in the optimum temperature for fixation between serums, both kinds of serums can be detected. This is done by Kaliski, who uses two hours icebox, followed by a half hour thermostat, incubation.

#### THE ANTIGEN

The most disputed point in the whole technic of the Wassermann reaction has been the antigen, the question of which particular type of antigen is best, and how it should be used. It is practically impossible to come to any conclusion on this question from the perusal of the literature. Different workers have worked with such different technic that in most instances no direct comparison of their results is possible. Probably no agreement can be reached until a more standard and uniform technic is used in comparing the different antigens. The first point for us, therefore, is to try to establish the best mode of using antigen in general.

*Considerations Concerning the Dosage of Antigen.*—Since the Wassermann reaction is a quantitative reaction, naturally the prime question with any antigen is how to ascertain the correct amount of it to use in the test. In general, it is true of any antigen that the greater the amount one can use of it, the more delicate the test, but that the nearer one approaches the amount that begins of itself to become anti-complementary, the greater the danger of nonspecific fixations. There seems to be an exception to this rule in the as yet unconfirmed observation of L'Esperance and Coca,<sup>31</sup> that with the acetone insoluble lipoid

30. Altman: *Dermat. Ztschr.*, 1912, **19**, 22; *Arch. f. Dermat. Syph.*, 1912, **111**, 871.

31. L'Esperance and Coca: *Jour. Immunol.*, 1916, **1**, 129.

fraction there is a prezone, that is to say, that when the tests are done in the particular way in which Coca does them, if the antigen is increased beyond a certain point, one fails to get a positive reaction with some serums.

In experimenting to determine the correct dose of antigen to use for Thomsen's or Thomas and Ivy's methods of doing the reaction, I have come across certain facts, which, while not suggesting a prezone, indicate that the larger dose of antigen does not necessarily give a positive reaction with a weaker reacting serum than does a smaller dose of antigen, in each instance the complement dose being so adjusted that no anticomplementary effect is to be feared from the antigen. It will be remembered that in these methods (those of Thomsen, Boas, Thomas and Ivy) the dose of antigen recommended was quite arbitrary and the complement dose was adjusted to the antigen in each case. It seemed to me in working with these methods that the arbitrary dose chosen might not be the optimum one. Experiments were therefore made with larger doses with the surprising result that when the complement dose was enlarged sufficiently to allow for the larger anticomplementary effect of the larger dose of antigen, complement fixation was not obtained with so high dilutions of a positive serum as when a smaller dose of antigen was used. The reverse experiment was therefore tried, namely, the use of smaller doses of antigen than the arbitrary ones usually given, and it was found that actually somewhat better results could be obtained with smaller doses, namely, fixation in higher dilutions of the positive serum. For this reason I am now using an amount about half as large as that recommended by Thomsen and by Thomas and Ivy.

The probable explanation of these observations is that when the dose of antigen is increased beyond a certain point, the amount of complement which has to be added to overcome its anticomplementary effect is too great to be fixed by certain grades of positive serum, or, in other words, that beyond a certain point the anticomplementary effect of antigen increases more rapidly than its antigenic effect. This suggests a method of determining the optimal dose of each new antigen for the Thomsen or Thomas and Ivy or the Browning and McKenzie methods. This method will be discussed below. The experiments on which it is based are described in a separate publication.<sup>32</sup>

Wassermann himself laid down the rule that the dose of antigen should be one half of the largest amount which itself was found to be not at all anticomplementary; and the tests were not to be read until the control tube containing double the dose of antigen used in the tests

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32. Ottenberg: Methods of Determining the Optimum Amount of "Antigen" for the Wassermann Reaction, *Jour. Immunol.*, to be published.



showed complete laking. It would seem hardly necessary to point out today that in doing the titration to determine the nonanticomplementary dose it is necessary to first incubate the antigen complement mixtures for the same length of time and at the same temperature as the preliminary incubation in the test proper, and only then to add sheep cells and amboceptor. With a good antigen of the simple alcoholic extract type the amount prescribed by Wassermann usually contains at least five to ten antigenic units, that is, it is at least five to ten times as large as the smallest dose which gives complete fixation with a positive serum.

The rule of one half the nonanticomplementary dose has been departed from by probably the majority of subsequent workers, apparently without a very good reason. Thus, with alcoholic extracts, MacIntosh and Fildes use one third of that amount, which alone is completely anticomplementary; Kaliski uses from one third to one fourth of a dose which shows beginning anticomplementary effect; the New York health department uses from one third to one fourth of a dose which shows no anticomplementary effect. With cholesterinated antigens likewise there is the widest divergence in the method of deciding the dose; thus Field specifies one fifth of the dose which begins to show anticomplementary power, Walker and Swift one fourth of the dose which is completely anticomplementary, Sachs a half of a dose which itself is not at all anticomplementary.

An entirely different principle is used by those who follow the method of titrating the complement (Thomsen, Boas, Thomas and Ivy, Browning and McKenzie) for each day's work in the presence of the antigen. All these workers use fixed and purely arbitrary doses of antigen, adjusting the dose of complement to that of antigen in such a way that the amount of antigen used is not one half or smaller fraction of the nonanticomplementary amount, but actually the largest non-anticomplementary dose itself; thus, double the amount of antigen used is decidedly anticomplementary. In fact, Boas states that his antigen should be completely anticomplementary in the double dose. The adjustment is not of antigen to complement, but of complement to antigen. In making this adjustment the fixed dose of antigen and varying doses of complement (and in the methods of Browning and McKenzie and of Thomas and Ivy, also pooled negative serum) have to be first incubated under precisely the same conditions as in the preliminary incubation of the test itself. The fixed dose used by Thomsen and by Boas is 0.2 c.c. of the alcoholic heart extract, that used by Stern 0.3 c.c. of the alcoholic heart extract, by Sormani from 0.25 c.c. to 0.3 c.c., by Thomas and Ivy 0.2 c.c. of alcoholic extract of syphilitic liver, by Browning and McKenzie 0.2 c.c. of cholesterinated alcoholic beef heart extract.



There does not seem to be a fixed principle on which the selection of this arbitrary dose is based except the principle of empirical experience, the fact that one obtains satisfactory results with the dose used. All of these workers used doses which, compared with those used in the original Wassermann system, are large, but the hemolytic system is adjusted to the dose of antigen. Though the larger dose superficially seems to have the advantage of providing a greater multiple of antigenic units than the smaller dose, this is not actually the case. And (as is pointed out in a separate communication) the dose should not be arbitrary, but should be determined accurately by ascertaining which one of a series of possible doses of antigen, when combined each with its appropriate dose of complement, gives complete fixation with the highest dilution of a positive serum.

To summarize this important subject of dosage of antigen, there are two schools of workers, those who adjust the dose of antigen to the complement and those who adjust the dose of complement to the antigen.

In the former school, comprising such workers as Wassermann, Citron, Sachs, Walker and Swift, Noguchi, and New York department of health, the standard by which the dose of antigen is determined is the anticomplementary effect; but the method of adjusting the dose according to the anticomplementary effect varies widely. It is suggested that in the interest of uniformity all workers who follow this plan should adhere to Wassermann's original standard, the dose of antigen which of itself, after preliminary incubation, is not at all anticomplementary. This dose, in my experience, is more constant and easily determined than the dose which begins to be anticomplementary or the dose which is just completely anticomplementary. Which particular fraction, one half, one fourth, one sixth, or what not, of this nonanticomplementary dose should be chosen, is a matter of empirical determination and has to be settled for each type of antigen separately. Probably a majority of the workers who use simple alcoholic extracts or acetone insoluble lipoids would agree on one half of the nonanticomplementary dose. With cholesterinated antigens it is generally recognized that a smaller fraction is necessary. Boas<sup>33</sup> pointed out in 1913 that if the usual rules of dosage were applied to cholesterinated antigen nonspecific results were frequent. It is possible that some of the unfavorable reports on cholesterinated antigens in the literature are based on dosage relatively too great.

The second school of workers, who use an arbitrary dose of antigen and adjust the dose of complement to it comprise such workers as

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33. Boas: The Wassermann Reaction, Ed. 2, 1913.

Browning and McKenzie, Thomsen, Boas, Sormani, M. Stern, and Thomas and Ivy. This method seems to me at least as good as the preceding. But the dose of antigen should not be arbitrary, because for each antigen there is an optimal dose. And this optimal dose is easily determined by trial of each proposed dose with a series of dilutions of a positive serum.

The method by which the alcoholic antigen is diluted with saline solution is of some importance. Sachs and Rondoni<sup>34</sup> in 1909 showed that by fractional dilution, that is, by the slow addition of alcoholic antigen to saline solution, a more opaque emulsion and in some instances a stronger antigen was obtained than by rapid addition of saline to antigen or of antigen to the saline solution. In my experience, while the slow dilution sometimes does give a smaller minimal antigenic dose, that is, a smaller amount is needed to give a positive result, it also makes the antigen anticomplementary in a smaller dose, and therefore the value of the antigen (the ratio of the two) remains the same. The important thing, therefore, is not which method of dilution (slow or rapid) is used, but that whichever method is used should be adhered to uniformly throughout.

*The Choice of Antigen.*—The great majority of workers today believe that the aqueous and alcoholic extracts of syphilitic organs need no longer be seriously considered in the practical performance of the Wassermann reaction. There are at present three types of lipid extracts that have received general acceptance. Although it seems quite possible that of the many other lipid preparations which have been recommended for the Wassermann reaction some may be quite as good or even better than these three, we can limit our consideration to them, namely, to the simple alcoholic extract of heart muscle, the alcoholic extract purified by acetone precipitation after the method of Noguchi, and the alcoholic extract reinforced by cholesterin according to the recommendation of Sachs, Walker and Swift, and others.

In spite of the enormous amount of work which has been done on the subject, it is impossible to draw final conclusions as to which type of antigen is best. I have used simple alcoholic extracts of beef and of guinea-pig heart, acetone insoluble fraction of beef heart, cholesterin reinforced guinea-pig heart, and cholesterin reinforced human heart. The simple alcoholic extracts from the different species of heart muscle seem to be practically identical in action, although there are rare cases in which a serum gives a positive reaction with one species and not with another. Personally, I have come to rely on beef extract because it is the easiest to prepare and extremely uniform in titer.

Although in my hands the acetone insoluble fraction has not shown any decided superiority over the simple alcoholic extracts, I have not

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34. Sachs and Rondoni: Ztschr. f. Immunitätsforsch. u. exper. Therap., 1908-1909, **1**, 132.



used it in parallel tests extensively enough to offer a definite judgment as to its value.

The use of cholesterin reinforced alcoholic extract is a vexed question. I have used this antigen in over 1,500 tests in which at the same time the reaction was done with simple antigen. Of these, the first 250 will be disregarded, as I am inclined to consider the rather high proportion (10 per cent.) of disagreements obtained in them as due partly to my inexperience with the method used (that of C. W. Field).

In the last 1,241 tests the method used has been that followed by Walker and Swift, and I have had the invaluable assistance of Dr. E. C. Jaegle, who has been using this method for years. The only change in the method has been the addition of an extra tube containing the half dose of patient's serum and the half dose of antigen, so that the quantitative readings (four plus, three plus, two plus and one plus) could be made according to the scheme of Citron, given farther along in this paper.

If the weak inhibitions (plus and two plus) are disregarded, the two antigens gave identical results in 1,174 cases, 95 per cent. of the total. Of the sixty-seven disagreements, forty-two gave definite positive reactions (four plus and three plus) with cholesterin and negative or doubtful reactions with simple antigen. Of the forty-two, ten were cases of undoubted syphilis and ten were doubtful cases, in which syphilis could not be excluded clinically.<sup>35</sup> Twenty-two were cases in which there was no reason to suspect syphilis. Of this twenty-two, eighteen were cases of pregnancy (7 per cent. of the 244 pregnancy cases examined).

On the other hand, twenty-five of the sixty-seven disagreements were cases in which cholesterinated antigen gave negative, simple alcoholic extract positive results. Twenty of these were known or highly probable cases of syphilis, five were doubtful cases in which syphilis could not be excluded.

It must be confessed, however, that if weak inhibitions (plus and two plus) are taken into account, cholesterinated antigen, though it gave a far greater number of weak positive reactions in treated syphilis, also gave a considerable number of false positive reactions in cases surely not syphilitic.

From this experience I venture to draw the following conclusion: Cholesterin reinforced antigen should never be relied on alone for diagnosis; weak inhibitions with it should not be given great weight excepting in known cases of syphilis; positive results obtained with it

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35. Most of the cases were ambulant and the clinical diagnoses were those of the physicians in charge of the various departments of the Vanderbilt Clinic, the Sloane Hospital for Women, and the Knapp Memorial Hospital for Eye Disease.



alone in pregnancy should be disregarded. On the other hand, in following the progress of cases of syphilis under treatment, the cholesterinated antigen is of very great value because it continues in many cases to give weak positive reactions long after the reaction to simple antigen has disappeared; and the opinion of syphilographers is that treatment should be continued until the reaction to cholesterinated antigen is also expunged. On the other hand, cholesterinated antigen should not be relied on alone in these cases either; for, as the author's experience shows, there is a not inconsiderable number of cases of known or treated syphilis in which a positive result is obtained with simple, but not with cholesterinated, antigen.

#### QUANTITATIVE READINGS OF THE WASSERMANN REACTION

In recent years the demand has more and more been made that the result of the Wassermann test be not merely reported as positive or negative, but that the strength of a positive reaction be indicated. This is of particular value in observing the result of treatment. A few workers have satisfied themselves by merely judging the strength of the reaction from the degree of inhibition in a single tube; but it is apparent that this method is extremely inaccurate, and various methods of titrating the strength of the reaction have been proposed.

Beyond a doubt great confusion has been caused in the medical mind by the divergent systems used by different workers for discovering and reporting the strength of reactions. This is one of the chief causes of the present lack of confidence in the Wassermann reaction. For this reason, as well as for its intrinsic importance, the subject deserves special attention.

Since the Wassermann reaction is a reaction between three different substances, antigen, patient's serum, and complement, it is evident that the reaction may be reduced by progressive steps to the vanishing point by using progressively diminishing (or in the case of complement progressively increasing) amounts of any one of these three substances. As a matter of fact, all three methods, that of using diminishing amounts of patient's serum, or increasing amounts of complement, or diminishing amounts of antigen, have been proposed and used with success by different workers for this purpose.

Citron, who was one of the first to introduce quantitative methods into the practice of the Wassermann reaction, employs two of the principles, that is, he uses simultaneously descending doses of both antigen and patient's serum. He uses only two tubes, the first tube containing the full dose of antigen and serum, the second half the dose of each, and he makes readings as follows:

Tubes 1 and 2 show complete absence of hemolysis	++++	} Strongly positive
Tube 1 shows complete absence of hemolysis, and Tube 2 shows faint hemolysis.....	+++	
Tube 1 shows complete absence of hemolysis, and Tube 2 shows complete hemolysis.....	++	} Weakly positive
Tube 1 shows partial hemolysis, and Tube 2 shows complete hemolysis.....	+	
Tube 1 shows doubtful binding, and Tube 2 shows complete hemolysis .....	±	} Doubtful Negative
Tubes 1 and 2 show complete hemolysis.....		

Readings made in this way have the advantage of indicating four different degrees of positive reaction with the addition of only one extra tube. And if for the time being one takes it for granted that to use half the dose of antigen gives the same result as to use half the dose of serum, the four grades may be taken to correspond roughly to four, three, two and one units of positive reaction.

It should be pointed out that there are many workers at present who in their reports use Citron's scheme of notation (four plus, three plus, two plus and one plus), but without using Citron's method of reading the strength of the reaction. This is done even by many who depend on the appearance of a single tube for determining the strength of the reaction; and this practice has led to many apparently inconsistent reports.

Thomsen and his pupil, Boas, use the method of descending doses of patient's serum; in the tubes in which there is incomplete inhibition they read the percentage of laking by comparing the color after sedimentation of the cells with the color of a hemoglobin scale (the hemoglobin scale is made by laking the same amount of red cells as used in the test, and of this laked mixture preparing successive percentage dilutions with water). They only report positive in any given tube if there is over 20 per cent. of inhibition according to this scale, and they express the strength of the reaction on a numerical scale. This method has been followed by many other workers. MacIntosh and Fildes, who also employ descending serum doses, believe that they can show that descending amounts of antigen give relatively abrupt, while descending doses of patient's serum give relatively gradual, changes. This needs confirmation.

I have used the Thomsen method (descending doses of patient's serum, 0.2 c.c., 0.1 c.c. and 0.05 c.c.) in approximately 1,000 tests, parallel with the Citron method, and have found a fairly close correspondence. Fixation with full dose, half dose and quarter dose of serum correspondingly roughly to one plus, two plus and four plus in the Citron scale.

Sormani in 1911 appears to have introduced the method of using descending doses of antigen. He uses five doses, of which the smallest is the least dose that gives complete inhibition with a very strong



secondary syphilitic serum, and the other five are successive multiples (2, 3, 4 and 5 times) of this. He reads his results 0.2, 0.4, 0.6, 0.8, 1 positive according as one, two, three, four or five of the tubes show complete inhibition. A good many other workers have adopted this method; for example, Field uses six arbitrary doses of antigen and has a rather more complicated numerical system for expressing the results.

The third method, that of using increasing units of complement, seems to have been introduced by Browning and McKenzie and has been adopted by Thomas and Ivy. As explained above, in the section on the adjustment of complement, Thomas and Ivy titrate complement in the presence of antigen and negative serum and adjust the dose in such a way that one free unit of complement is present in the main test. In successive tubes, then, they set up tests with the same amount of antigen and patient's serum and containing two, three, four or more free units of complement.

I have adopted the method of Thomas and Ivy and used it extensively. Two extra tubes are set up, the one containing two, and the other four free units of complement. It should be explained that the measuring of two and four free units of complement in these tubes is attained, of course, not by using two and four times the amount of guinea-pig serum found necessary in the first tube in the titration, but by adding to the amount in the first tube the unit of complement as determined in a separate titration (in which antigen and normal serum are not present). (See below.)

In approximately 5,000 tests I have used this method of ascending units of complement, parallel with the Citron method of descending doses of antigen and serum, and have found that one, two, three and four plus, according to the Citron method, in the great majority of cases correspond closely with the power to fix one, two, three or four units of complement in the Thomas and Ivy method.

I have also experimented with the method of Browning and McKenzie, using cholesterin reinforced antigen and ascending units of complement. From the point of view of accuracy this method is ideal, since it shows exactly how many units of complement are fixed by the reaction.

With this method anticomplementary serums cause no difficulty, since the anticomplementary effect of each serum (as well as that of the antigen) is measured directly in units of complement, and subtracted from the number of units of complement fixed in the test. For the same reason natural antishoop hemolysin plays practically no rôle because its effect in the control series is directly subtracted from the main series. I have found, however, that the amounts of complement specified in Browning and McKenzie's communications are far too



large to detect any except very strongly positive serums; and I have come to use three, five and eight units instead of seven, ten, fifteen, twenty, thirty and forty, as advised by Browning and McKenzie. The only objection to the method is that it is too elaborate for routine work with a large number of tests. In the simplified form used by me, however, it requires only six tubes for each test; on account of the superior opportunities for accuracy it offers it is worthy of a wider use than it seems to have had.

From my trial of all these methods I am convinced that the present status of the Wassermann reaction would be greatly improved if any one of these ways of making quantitative estimations could be adopted by all workers, and it would not make a great difference which one was adopted. The methods, however, which offer special advantages for standardization are those (Browning and McKenzie, Thomas and Ivy) which depend on the principle of measuring the number of units of complement fixed, that is, measuring the work of complement fixation directly in the amount of work done. I regard complete fixation of one free unit of complement with simple alcoholic antigens and of two free units with cholesterolin reinforced antigens as the minimal definitely positive reaction.<sup>36</sup>

Since Citron's terminology, one, two, three and four plus has impressed itself deeply on the popular medical mind it had better be preserved, one plus representing the weakest definitely positive grade as defined above and four plus the strongest.

#### MINOR TECHNICAL CONSIDERATIONS

The strength of the saline solution used throughout the test varies in practice of different workers from 0.85 per cent. to 0.9 per cent. Probably it makes no difference which of these two strengths is used provided the same strength is adhered to uniformly. It is of real importance that the solution be made with accuracy, however, and occasionally disasters which occur in the laboratory are traced to errors in this step.

The water used is of course distilled water, and as this contains carbon dioxide absorbed from the air, it is the practice of some laboratories (New York health department) to boil the saline and then add tenth-normal potassium hydroxid solution until a sample gives a faint pink with phenolphthalein. As all the ingredients of the test are exposed to the air before and during the test, however, this seems an unnecessary refinement.

Absolute cleanliness of the glassware is a thing of great importance and occasional discrepancies and errors which occur (and which of course should be easily detected if the proper controls are used) are traced to foreign substances adhering to the tubes. The method of cleaning test tubes used at the New York health department laboratory is the best I have seen. The

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36. The mode of measuring the unit of complement must of course be agreed on. I have throughout my work titrated the complement unit with two units of amboceptor and I have used this fixed dose of amboceptor in all tests. The principles laid down hold good, however, whether this particular method of titration is adopted or not.

tubes, packed firmly in copper baskets, are rinsed in tap water, and boiled in soap water; the alkali of the soap water is neutralized by dipping the baskets in dilute hydrochloric acid, after which they are immersed in running tap water and dried in ovens.

It has been claimed<sup>37</sup> that the alkalinity of new glassware may lead to false positive tests. This has never occurred in my experience. Nevertheless it seems wise thoroughly to boil new glassware in distilled water.

The test tubes used must be fairly uniform in caliber. Hemolysis is distinctly delayed in very narrow tubes, possibly due to imperfect mixing of the ingredients.

The use of preserved complement has the advantage that the mixed complement from a large number of guinea-pigs can always be used. Two methods of preserving complement are in vogue—salting<sup>38</sup> (addition of from 8 to 25 per cent. of sodium chlorid; dilution with distilled water before use) and freezing. To judge from the literature both methods are satisfactory. Stern<sup>39</sup> claimed that frozen complement lost some of its binding power, though not its hemolytic power; this has had no recent confirmation and should be reinvestigated. I have used salted complement and found it satisfactory. The salted complement must, however, be kept at a temperature very near 0 C. or it gradually deteriorates.

#### GENERAL PRECAUTIONS FOR ELIMINATION OF ERROR

It is impossible to discuss here all of the elements which enter into an accurate and safe technic, but there are a few special points, not so generally observed as they should be, that deserve special discussion. One of these is the doing of all tests as well as all titration in duplicate. The Wassermann reaction is a quantitative reaction. In its essence it is probably also a chemical reaction. No chemist would think of relying on a single quantitative determination in an important case. He would make a duplicate, and if the two determinations did not agree, he would make another estimation. Accurate results with the Wassermann test are more rather than less difficult to obtain than are accurate results in purely chemical work. For this reason, in spite of the increased labor, all titrations as well as all tests ought to be done in duplicate.

It is advisable in duplicate tests to vary the technic slightly so as to get the advantage of seeing from two different angles—binocular vision. Many workers do this by employing two or more different antigens. As has been pointed out in the foregoing, differences in results are due quite as often to variations in technic as to differences in antigen. For this reason different technics as well as different antigens should be employed, at least until some one system has been shown to be greatly superior to all others. At present I make all tests by three methods, as follows:

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37. Sternberg: *Wien. klin. Wchnschr.*, 1914, **27**, 545.

38. Austin: *Jour. Am. Med. Assn.*, Correspondence, 1914, **62**, 868. Thompson: *The Preparation and Preservation of Complement*, New Instruments and Suggestions, *Jour. Am. Med. Assn.*, 1916, **66**, 652.

39. Stern: *Berl. klin. Wchnschr.*, 1908, **45**, 1489.



1. A modified Thomas and Ivy method with simple alcoholic beef heart extract; preliminary incubation four hours in the icebox; use of two extra tubes with increasing doses of complement (two and four units) for determining the strength of the reaction; one extra serum control tube with two units of complement;<sup>40</sup> final addition of cells and two units of amboceptor.

2. A modified Citron method with simple alcoholic extract, icebox incubation, two units of complement (the unit determined with two units of amboceptor), Kaliski's device (see above) for detecting and compensating for natural antisheep hemolysin, final addition of two units of amboceptor when needed.

3. Cholesterin reinforced antigen according to the method employed by Walker and Swift, slightly modified, and with the addition of Citron's tube for determining the strength of the reaction; two units of complement in the "main" tubes, one unit in the serum control tube (the unit is determined with one unit of amboceptor and hence is larger than in the modified Citron method described above); two units of amboceptor; preliminary incubation in water bath at 37 C., and final addition of sensitized sheep cells, without attention to natural amboceptor.

Another precaution that should be taken much more often than it is, is the checking up of results with the results of another laboratory. Every laboratory should have an arrangement with at least one or two other laboratories for the regular and frequent interchange of serums on which the results can be compared.

Of even greater importance is the clinical checking up of the cases. In reading the results of the tests the laboratory worker should pay no attention to the clinical diagnosis. But after the results have been read objectively and recorded they should all and invariably be compared with the clinical diagnoses. Many clinicians do not realize the importance of this, and harbor unfair suspicions of the laboratory man when he insists on knowing the clinical diagnoses. But as a matter of fact, since the Wassermann reaction is a test which has no absolute, but only a practical, specificity, it is necessary constantly to check up the results by a knowledge of the clinical diagnoses. It sometimes happens, for instance, that with this knowledge on a particular date the laboratory worker is able to recognize the fact that all his tests are "weak," that is to say, due to some inadvertent variation in technic, such as the use of too large a dose of complement, some weakly positive serums have not been detected. This being known, he can then repeat the tests with proper precautions.

A further control on the constancy and delicacy of the reaction in detecting weak positive results is the use of a weakly positive control serum, as introduced by Citron. This consists of setting up, in addition to the usual control test with a known strong positive serum, another test, using a serum known from previous testing to be weakly positive. If no such serum is on hand, a strongly positive can be diluted four to eight times. It has to be remem-

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40. This is provided in case the first serum control tube containing only one unit of complement fails to lake completely. If this happens then the first "main" tube is disregarded and readings made only from the tubes containing two and four units of complement.



bered, of course, that positive serums become weaker on standing for more than a few days unless special precautions are taken to preserve them.

The antigen control test is, of course, absolutely essential, no matter how often or how recently one has used an antigen or how great one's confidence is in its reliability. This is particularly true since a rare occurrence is the finding of a guinea-pig whose serum gives a positive Wassermann reaction. I have seen this once only in several thousand of guinea-pigs used in the last seven years. Dr. Jaegle has seen it twice.

#### CONCLUSION

Divergent reports on identical serums sent to different laboratories undoubtedly occur and will continue to occur so long as laboratory workers continue to use widely different technical methods. These divergent results, however, should not shake our confidence in the clinical specificity of the Wassermann reaction. They almost invariably occur only in cases which exhibit weakly positive reactions, and they usually mean that one laboratory has succeeded in detecting a weakly positive reaction, while the other has not. In the great majority of cases which present definite positive or definite negative results the reports of different laboratories are practically uniform. The reason for the divergence in results on weakly positive cases is that some laboratories have adopted certain refinements of technic which other laboratories have for various reasons failed to adopt.

The original Wassermann technic, while safe in the sense of not giving false positive results, is not nearly so delicate in detecting weakly positive tests as it can be made. Many of the devices for making the reaction more delicate without impairing its safety are discussed and recommended in the present paper.

On the other hand there are a few workers who, in their anxiety to detect as many of the weakly positive cases as possible, have adopted methods which easily can and probably do lead to occasional false positive reports.

Though the technic of the Wassermann reaction is relatively simple to learn, the work is full of pitfalls and should be done only by properly trained workers and the results should be controlled by every possible control. While it does not seem possible that all workers will adopt one uniform technic, it is greatly to be desired that they should agree on basic principles and methods.

Though the present paper has discussed the Wassermann reaction only, the observations are nearly all applicable (with due control of specificity) to complement fixation with bacterial and other specific antigens.

## THE BACTERIOLOGY OF THE URINE IN FOCAL INFECTIONS; ITS RELATION TO NEPHRITIS \*

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In a previous report<sup>1</sup> the bacteriology of the urine in a number of cases of nonsuppurative nephritis was described. In all of the cases examined the urine contained numbers of bacteria, mostly anaerobes. In one case<sup>2</sup> the bacterial flora of the urine was the same as that of a focus of infection in another part of the body. The bacteria isolated from the urine readily produced experimental nephritis. These facts led us to believe that bacteria multiplying in foci of infection frequently gain entrance to the blood stream and are excreted in viable condition through the kidneys, and that the excretion of bacteria in this way by the kidneys may be a common cause of nephritis.

It seemed desirable to examine a number of patients with evident focal infections to learn how frequently bacteria found in the focus of infection can be isolated from the urine, in what number they occur in the urine, and whether excretion of bacteria occurs to any extent without clinical evidence of injury to the kidneys.

In selecting cases for this study, patients with gonorrhea, other primary infections of the urinary tract, or syphilis were excluded, so far as possible, by means of history, complement deviation tests, evident discharges, pus in the urine, vesical irritation, etc. Infections such as pneumonia, known to be accompanied by septicemia, were also excluded. No patient with evidence of an active tuberculosis was included. Some of the patients studied had evidence, such as arthritis, of migration and lodging of bacteria in other parts of the body. Cases in which the intestine seemed the most likely source of infection were avoided because of the proximity of the urethral and anal orifices, which might lead to the interpretation of correspondence between urinary and fecal flora as due to contamination rather than to transmission by the blood stream.

Eighteen patients were examined. In six of these the bacteria when found in the urine did not correspond to those found in the focus of

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1. Dick, G. F., and G. R.: The Bacteriology of the Urine in Nonsuppurative Nephritis, *Jour. Am. Med. Assn.*, 1915, **65**, 6.

2. Dick, G. F., and G. R.: The Bacteriology of the Urine in Two Cases of Parenchymatous Nephritis, *Jour. Am. Med. Assn.*, 1914, **63**, 1661.

infection from which cultures were made. This may have been due to the bacteria in the urine having come from a source other than the focus of infection studied. In some instances, the negative results may have been due to the time of examination, as it was found that showers of bacteria may occur in the urine just as showers of casts are known to occur. Indeed, the two were observed to appear together in Case 5, to be described. In most cases the bacteria were hard to grow in pure culture and difficulty of technic in isolating and identifying the organisms probably explains some negative results.

In four cases a partial correspondence was found between the bacterial flora of the urine and that of the focus of infection. In eight cases the correspondence was more striking. These eight cases will be described in detail.

Cultures from the focus of infection were made aerobically and anaerobically on human blood agar slants. Shake cultures in plain agar were made with 2 c.c. catheterized urine; 15 c.c. urine were centrifuged and aerobic and anaerobic cultures on human blood agar were made from the sediment.

#### REPORT OF CASES

CASE 1.—*Chronic tonsillitis, chronic interstitial nephritis, cardiac hypertrophy in a woman, aged 25.* History of frequent attacks of tonsillitis. Blood pressure 150 mm. The catheterized urine showed an occasional hyaline cast and leukocyte; no albumin or erythrocytes.

Cultures from 2 c.c. urine gave colonies too numerous to count of (a) streptococcus, oval, gram-positive aerobic, hemolytic, dextrose acid, mannite negative, lactose faintly acid, milk acid and not coagulated at the end of forty-eight hours; (b) bacillus, small, pleomorphic, gram-negative, anaerobic, no growth on ordinary mediums, no hemolysis, minute transparent colonies on human blood agar.

Cultures from the tonsils gave (a) streptococcus, oval, gram-positive, aerobic, hemolytic, dextrose acid, mannite negative, lactose faintly acid, milk acid, not coagulated at end of forty-eight hours; (b) bacillus, small, pleomorphic, gram-negative, anaerobic, no growth on ordinary mediums, no hemolysis, minute transparent colonies on human blood agar; (c) fusiform bacillus; (d) staphylococcus; (e) coccus, small, gram-negative, anaerobic.

CASE 2.—*Chronic rhinitis, pyelonephritis, post-operative bronchopneumonia, in a girl, aged 12.* History of "cold in head" all of preceding winter, frequent micturition for six months, tonsillectomy two months, and removal of inferior turbinate bones one week before being seen. Blood pressure 114 mm. The urine contained 0.2 to 0.8 gm. albumin to the liter; showed numerous leukocytes and some leukocytic casts, no erythrocytes.

Cultures of 2 c.c. urine gave fifteen colonies of (a) streptothrix, small, true branching, mostly gram-negative, growth in both aerobic and anaerobic cultures, no growth on ordinary mediums, very delicate, transparent growth of minute colonies on human blood agar, no change in the blood; one colony of (b) staphylococcus; one colony of (c) bacillus diphtheroid, gram-positive, aerobic.

Cultures of nasal secretion showed (a) streptothrix, small, true branching, mostly gram-negative, growth in both aerobic and anaerobic cultures, no growth



on ordinary mediums, very delicate, transparent growth on human blood agar, no change in the blood; (b) streptococcus, hemolytic.

CASE 3.—*Recurring bronchitis, chronic arthritis, nephritis, in a woman aged 49.* History of bronchitis every winter. Blood pressure 150 mm. Urine showed trace of albumin, occasional hyaline and finely granular cast, very few leukocytes, no erythrocytes.

Cultures of 2 c.c. urine gave one colony in shake, and several colonies on human blood agar slant, of (a) streptococcus, small spherical, gram-positive growth very scant both aerobically and anaerobically, forming minute, transparent colonies; hemolytic; lactose and dextrose acid, mannite negative, milk acid and coagulated; six small colonies of (b) bacillus, small, pleomorphic, gram-negative, anaerobic, no growth on ordinary media, minute transparent colonies on human blood agar, no change in the blood; one colony of (c) staphylococcus; two colonies of (d) bacillus diphtheroid, gram-positive, aerobic.

Cultures of sputum gave (a) streptococcus, small, spherical, gram-positive, growth very scant both aerobically and anaerobically, minute transparent colonies, hemolytic, lactose and dextrose acid, mannite negative, milk acid and coagulated; (b) bacillus, small, pleomorphic, gram-negative, anaerobic, no growth on ordinary mediums, minute, transparent colonies on human blood agar, no change in the blood; (c) pneumococcus.

CASE 4.—*Chronic tonsillitis, chronic interstitial nephritis, cardiac hypertrophy, in a woman aged 29.* History of tuberculosis at the age of 15; backache, headache, dizziness, dyspnea on exertion noticed during last year. No evidence of active tuberculosis; right tonsil hypertrophied, with tender swelling under angle of right jaw. Blood pressure 230 mm. Urine amounted to 870 c.c. in twenty-four hours; specific gravity 1.020; showed a trace of albumin and hyaline and granular casts.

Cultures of 2 c.c. urine gave colonies too numerous to count of (a) bacillus, small, pleomorphic, gram-negative, anaerobic, no growth on ordinary mediums, pin-point, transparent colonies on human blood agar, no change in the blood; (b) streptococcus, small, gram-positive, grew only in anaerobic blood slant and failed to grow in transfers; (c) leptothrix varying from short bacillary forms to long threads, gram-positive, anaerobic, three colonies obtained from 2 c.c. urine; (d) staphylococcus, one colony grew from 2 c.c. urine.

Cultures of tonsils showed (a) bacillus, small, pleomorphic, gram-negative, anaerobic, no growth on ordinary mediums, pinpoint, transparent colonies on human blood agar, no change in the blood; (b) streptococcus; (c) staphylococcus; (d) coccus, small, gram-negative, anaerobic; (e) bacillus, anaerobic, gram-positive, diphtheroid.

CASE 5.—*Chronic infection about root of an impacted tooth; chronic interstitial nephritis; cardiac hypertrophy.* History of diphtheria, scarlet fever and influenza. Complaint of headache and dizziness for one year. Blood pressure, 170 mm. Urine: 1,500 c.c. in twenty-four hours; sp. gr., 1.008; no erythrocytes or leukocytes, an occasional hyaline cast and no albumin on admission; later, a trace of albumin and a shower of hyaline and granular casts.

Cultures of 2 c.c. urine made on admission to hospital gave one colony of (a) bacillus, small, pleomorphic, not definitely gram-positive or gram-negative, anaerobic, no growth on ordinary mediums, pinpoint transparent colonies on human blood agar, no change in the blood.

Cultures of 2 c.c. urine made at the time the shower of casts appeared showed innumerable colonies of (a) bacillus obtained in first culture; (b) bacillus, fusiform, gram-negative, anaerobic; (c) coccus, very small, spherical, gram-negative, anaerobic, less growth on ordinary mediums than on human blood agar, no change in the blood, delicate growth of pinpoint transparent colonies;

(*d*) streptococcus, small, oval, gram-positive, anaerobic, hemolytic, small transparent colonies on human blood agar, dextrose and lactose acid, mannite negative, milk acid and not coagulated at end of forty-eight hours; (*e*) *Staphylococcus aureus*; (*f*) *Bacillus mucosus*.

Cultures of pus from infection around tooth showed (*a*) bacillus, small, pleomorphic, not definitely gram-positive or gram-negative, anaerobic, no growth on ordinary mediums, pinpoint transparent colonies on human blood agar, no change in the blood; (*b*) bacillus, fusiform, gram-negative, anaerobic; (*c*) coccus, very small, spherical, gram-negative, anaerobic, less growth on ordinary mediums than on human blood agar, no change in the blood, delicate growth of pinpoint transparent colonies; (*d*) streptococcus, small, oval, gram-positive, anaerobic, hemolytic, small transparent colonies on human blood agar, dextrose and lactose acid, mannite negative, milk acid and not coagulated at the end of forty-eight hours; (*e*) *Staphylococcus aureus*; (*f*) bacillus, very small, plump, gram-negative, anaerobic, hemolyzed blood slowly forming black colonies; (*g*) streptococcus, larger than (*d*) spherical, long chains, gram-positive, aerobic, heavier growth than (*d*) milk acid and coagulated at end of forty-eight hours.

TABLE GIVING DATA OF AUTHORS' CASES

Case No.	Clinical Evidence of Kidney Lesion	Organisms Found in Both Urine and Focus of Infection	Number of Bacteria in Urine
1	+	(a) Streptococcus..... (b) Anaerobic bacillus.....	Many
2	+	Streptothrix.....	Few
3	+	(a) Streptococcus..... (b) Anaerobic bacillus.....	Few
4	+	(a) Streptococcus..... (b) Anaerobic bacillus.....	Many
5	+	(a) Streptococcus..... (b) Anaerobic bacillus..... (c) Fusiform bacillus..... (d) Anaerobic coccus.....	Few to Many
6	—	(a) Pneumococcus..... (b) Streptothrix.....	Many
7	?	(a) Streptococcus..... (b) Anaerobic bacillus.....	Many
8	+	Leptothrix.....	Many

CASE 6.—*Bronchiectasis (nontuberculous), in a woman aged 44.* History of cough and expectoration for three months following an attack of influenza. Blood pressure, 120 mm. Catheterized specimen of urine showed no albumin, casts, leukocytes or erythrocytes.

Cultures of 2 c.c. urine gave numerous colonies of (*a*) pneumococcus; (*b*) streptothrix, small, very pleomorphic, forming branched threads at times, at other times appearing as a very irregular, small bacillus, gram-negative, anaerobic, no growth on ordinary mediums, delicate transparent growth on human blood agar, no change in the blood; (*c*) bacillus, one colony only, of morphology of colon bacillus, gram-negative, aerobic, not motile, thick, moist, yellowish mucoid film on plain agar, no change in blood, lactose, milk acid and slowly coagulated, mannite and dextrose negative.

Cultures of sputum showed (*a*) pneumococcus; (*b*) streptothrix, small, very pleomorphic, forming branched threads at times, at other times appearing



as a very irregular small bacillus, gram-negative, anaerobic, no growth on ordinary mediums, delicate transparent growth on human blood agar, no change in the blood; (c) yeast.

CASE 7.—*Exophthalmic goiter, chronic tonsillitis, mitral stenosis and insufficiency, in a woman aged 25.* History of typhoid fever. Nervousness, dizziness, palpitation, diarrhea and one recent attack of nausea during past year. Blood pressure, 150 mm. Urine showed no albumin, casts, leukocytes or erythrocytes.

Cultures of 2 c.c. urine showed numerous colonies of (a) bacillus, small, regular, in morphology in direct smears of urinary sediment, pleomorphic in cultures, gram-negative, anaerobic, no growth on ordinary mediums, very delicate growth of pinpoint transparent colonies on human blood agar, no change in the blood; (b) streptococcus, marked tendency to pleomorphism with formation of long oval and bacillary forms, many of which are gram-negative, nonhemolytic.

Cultures of tonsils showed (a) bacillus, small, pleomorphic, gram-negative, anaerobic, no growth on ordinary mediums, very delicate growth of pinpoint transparent colonies on human blood agar, no change in the blood; (b) streptococcus, nonhemolytic, resembling that of urine; (c) streptococcus, hemolytic.

CASE 8.—*Chronic tonsillitis, acute rheumatism, aortic insufficiency, chronic nephritis, in a woman aged 25.* History of measles, whooping cough, tonsillitis, two attacks of rheumatism, dyspnea and palpitation. Blood pressure varied from 165 mm. to 190 mm. Urine showed albumin and coarse and fine granular casts, no leukocytes or erythrocytes.

Cultures of 2 c.c. urine gave about forty colonies of (a) leptothrix, thread-like forms, no branching, gram-positive, anaerobic, growth slow, forming minute, transparent colonies on human blood agar at end of forty-eight hours, which developed into slightly raised, brownish colonies about 1 mm. in diameter at end of five days, blood brownish, growth on dextrose, mannite lactose agar and in milk slight as compared with that on human blood agar.

Cultures of throat showed many colonies of (a) leptothrix of same characteristics as that of urine.

#### SUMMARY AND CONCLUSIONS

It was pointed out in our previous report<sup>3</sup> that staphylococci and gram-positive aerobic diphtheroids are common inhabitants of the urethra. Their occasional presence in small numbers as a contamination in catheterized urine is to be expected. They were found in Cases 2, 3, 4 and 5. It will be noted that aside from these organisms, one or more of the kinds of bacteria found in the urine were isolated from the focus of infection in each of the eight cases. It seems probable that the bacteria found in the urine came from the focus of infection.

It will be seen from the table that two of the eight patients showed no clinical evidence of renal lesion. One of these, however, had persistently high blood pressure (150 mm.). In the remaining patients there were from mild to severe renal lesions present, but there was no very constant relation between the severity of the lesion and the number of bacteria in the urine.

3. Loc. cit., Note 1, p. 15.



The finding of considerable numbers of bacteria in the urine in 66 per cent. of patients with evident foci of infection indicates that in diseases where migration of bacteria from foci of infection takes place, the bacteria are often present in the urine.

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## THE EFFECT OF MUSCULAR WORK, DIET AND HEMOLYSIS ON THE SERUM PROTEINS

TOGETHER WITH COMMENT ON THE TECHNIC AND CLINICAL USEFULNESS OF ROBERTSON'S MICROREFRACTOMETRIC METHOD \*

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### MUSCULAR WORK

Böhme<sup>1</sup> has shown that muscular work, even of the simplest sort, increases the serum concentration. Schwenker<sup>2</sup> found this concentration returned to a constant normal level after the patient had reclined quietly for twenty minutes. His explanation of the increase was that during work, blood pressure rises, which forces protein-poor fluids out of the blood vessels into the surrounding tissues. Reiss,<sup>3</sup> on the other hand, thought that during activity muscles take up considerable water, giving it up during rest.

Our investigations on this effect of work on serum proteins in normal men show a very decided increase in the total proteins, which increase occurs more in the albumin than in the globulin fraction. The nonproteins increase very slightly. The work done was on a bicycle ergometer to a point where the subjects were near exhaustion, except in Case 3, in which the increase in proteins due to merely walking around the laboratory is shown. On two cases, the degree of acidosis due to work was estimated by Van Slyke's recent method. Thus, these results confirm former work with the refractometer on this subject and show the manner in which this increase is split between the serum albumin and globulin as well as the effect of work on the nonproteins.

### FOOD AND FLUIDS

Böhme<sup>1</sup> and Reiss<sup>3</sup> have shown, by the refractometer, that the concentration of serum is only slightly changed after a large meal, this change following no rule, being either an increase or decrease. We

\* Submitted for publication Aug. 29, 1916.

\* From the Medical Service and Chemical Laboratory of the Massachusetts General Hospital, Boston.

1. Böhme: *Deutsch. Arch. f. klin. Med.*, 1911, **103**, 522.

2. Schwenker: *Inaugural Diss.*, Würzburg, 1905.

3. Reiss: *Ergebn. d. inn. Med. u. Kinderh.*, 1913, **10**, 531.

have used Robertson's microrefractometric method<sup>4</sup> to reinvestigate this problem, taking the serum from one to one and one-half hours after the test meal, during which period Böhme showed slight changes in the serum concentration. The test meal was high in carbohydrates and protein. Little fat was given, so that a lipemia would not interfere with the refractometric readings. Our results show either a slight increase or slight decrease in the serum proteins, which agrees with former findings. Serum albumin changed more than globulin, except in Case 5. Nonproteins were not changed except for 0.1 per cent. in Case 1.

The effect of high protein diets given over a number of days on serum proteins was investigated refractometrically by Chajes,<sup>5</sup> who obtained slight increases in some cases with stationary values in others.

TABLE 1.—EFFECT OF WORK ON SERUM PROTEINS IN NORMAL MEN

Case No.	Serum Proteins Before Work*				Work Done in Kilo-gram-meters	Serum Proteins After Work				Increase in Proteins Due to Work				Volume Percentage of CO <sub>2</sub> Bound by Blood Plasma after Work†
	Albu-min	Glob-ulin	Total Pro-tein	Non-pro-tein		Albu-min	Glob-ulin	Total Pro-tein	Non-pro-tein	Albu-min	Glob-ulin	Total Pro-tein	Non-pro-tein	
1	5.9	1.6	7.5	1.3	16,995	6.7	1.9	8.6	1.4	0.8	0.3	1.1	0.1	....
2	5	1.7	6.7	1.3	Slight exercise with walking	5.3	1.9	7.2	1.3	0.3	0.2	0.5	0	....
3	5	1.7	6.7	1.3	11,360	6.5	2.1	8.6	1.3	1.5	0.4	1.9	0	41.9
4	6	1.5	7.5	1.3	14,797	7	2.1	9.1	1.35	1	0.6	1.6	0.05	48

\* Serums were taken from patients who had been lying down for at least twenty minutes.

† Van Slyke's method.

Our results show that after four days of high protein diet there is on the average a slight increase in the total proteins, and we found no definite division of this increase between the albumin and globulin fractions. The nonproteins were slightly increased in three cases, decreased in one case, remaining stationary in two cases.

As a result of this work on the effect of diet on serum proteins, it seems that an immediate effect is practically wanting, while a long continuance of high protein diets slightly increases the serum proteins.

In this connection it is worth while noting that Engel and Scharl<sup>6</sup> and Reiss<sup>3</sup> found no definite sort of variation in normals or in heart or kidney cases at any time after drinking large amounts of water or

4. Robertson: Jour. Biol. Chem., 1915, **22**, 233.

5. Chajes: Therap. d. Gegenw., 1904, **45**, 442.

6. Engel and Scharl: Ztschr. f. klin. Med., 1906, **60**, 225.



milk. Markus<sup>7</sup> found a slight increase in the serum concentration of a chronic nephritic as a result of drinking water. On the other hand, the refractometric work of Benczur<sup>8</sup> and others has shown that the concentration of serum does decrease when extra salt is taken into the system, this decrease disappearing soon in normal people, but persisting for long periods when kidney damage is present.

#### VARIATIONS ASSOCIATED WITH NONSEPTIC HEALING

We have noticed that the percentage of globulin has been rather high when fractures or postoperative hernial incisions were healing. In only one case, though, was this percentage above normal limits. These percentages in healing fractures were 39, 31, 27, 30, 27.5 and 30, while in healing herniotomy incisions they were 31, 29, 29. These patients were all confined to bed and were running no fever, and no septic foci were present.

TABLE 2.—IMMEDIATE EFFECT ON SERUM PROTEINS BY FOOD

Case No.	Serum Proteins Three Hours After Breakfast				Caloric Intake of Test Meal				Serum Proteins 1 or 1½ Hours after Test Meal				Variations in Serum Proteins Due to Food			
	Albu-min	Glob-ulin	Total Pro-tein	Non-pro-tein	Pro-tein	Fat	Carbo-hy-drates	Total Calo-ries	Albu-min	Glob-ulin	Total Pro-tein	Non-pro-tein	Albu-min	Glob-ulin	Total Pro-tein	Non-pro-tein
1	4.8	2	6.8	1.3	185	39	530	754	4.9	2	6.9	1.2	0.1	0	0.1	-0.1
2	5.6	1.8	7.4	1.2	126	28	449	603	5.2	1.7	6.9	1.2	-0.4	-0.1	-0.5	0
3	4.6	1.9	6.5	1.25	180	38	510	728	4.5	1.9	6.4	1.25	-0.1	0	-0.1	0
4	4.6	2.1	6.7	1.2	185	39	464	688	4.8	2.2	7	1.2	0.2	0.1	0.3	0
5	6.5	1.7	8.2	1.3	*	*	*	*	6.4	1.4	7.8	1.3	-0.1	-0.3	-0.4	0

\* Meal similar to those above.

Though these values are within normal limits, except in one case, the repeated finding of results in the upper limits of the normal variation is probably of significance. It seems possible that this elevation above the normal average of about 24 per cent. might be due to the effect of toxic split proteins liberated in the healing processes. It is assumed that such toxic substances increase the globulin, in the same way that nonspecific protease or vaccines produce leukocytosis and undoubtedly raise the globulin, as shown in recent articles.<sup>9</sup>

#### CHANGES IN SERUM PROTEINS DUE TO KEEPING SERUMS ON ICE FROM TWENTY-FOUR TO FORTY-EIGHT HOURS

Table 4 demonstrates that if serums are kept on ice, they can be examined at any time within forty-eight hours after the blood-letting

7. Markus: Berl. klin. Wchnschr., 1907, **44**, 506, 537.

8. Benczur: Ztschr. f. klin. Med., 1909, **62**, 164.

9. Jobling and Petersen: Jour. Am. Med. Assn., 1916, **66**, 1753.

without fear of changes in the serum proteins. In Cases 10 and 11 the serum was left in contact with the clot, which made no important difference in the results. The variations present are nearly within the limits of error of this technic.<sup>10</sup>

#### HEMOLYSIS

The effect of hemolysis on serum proteins is shown in Table 5. In Case 2 it was hemolyzed as much as possible by repeated freezing and thawing without preventing its reading with a sodium flame. The increase in total protein is considerable, the increase in the albumin being either greater than or equal to that in the globulin. There is also a slight increase in the nonproteins. The gradual decrease in the refractometric reading as hemolysis of Serum 2 was increased is given in this place:

Degree of Hemolysis	Refractometric Reading
No hemolysis .....	64° 3'
Slight hemolysis .....	64° 1'
Moderate hemolysis .....	63° 55'
Limit of hemolysis permitting refractometric reading..	63° 45'

The slight amounts of hemolysis often present in serums only make a difference of a few seconds in the reading and thus practically no difference in the estimated serum proteins. If a slightly hemolyzed serum is perfectly easy to read with a sodium flame, the amount of increase in the serum proteins over the normal may be ignored except for the most accurate work. In our investigations we have used no serums that were hemolyzed except in a very few instances and then the hemolysis was always very slight.

#### TECHNIC

Throughout all our work on serum proteins the refractometric method as described by Robertson<sup>4</sup> has been followed. The comment on this technic here given may aid those who wish to use the method in further investigations.

As mentioned in a former article,<sup>11</sup> the syringe into which blood is drawn must be dry and clean so as not to dilute or change the blood serum. That article also shows the necessity of drawing blood from the vein within one minute after the tourniquet is applied so as not to increase the serum concentration by venous stasis.

The results of the present article show that in order to exclude the increase of serum proteins caused by any muscular activity, all patients should have been in a reclining position for at least twenty minutes

10. The limits of error due to technic in Robertson's microrefractometric method are about  $\pm 0.2$  per cent. albumin,  $\pm 0.15$  per cent. globulin, and  $\pm 0.1$  per cent. nonproteins.

11. Rowe: Jour. Lab. and Clin. Med., 1916, **1**, 485.

before blood is drawn. Though an immediate effect of food on serum proteins has been found to be practically wanting, in order to obviate lipemia, which makes the refractometric reading difficult, the taking of blood three hours after the last meal is desirable. Serums which are more than slightly hemolyzed should not be used. If inconvenient to estimate serum proteins immediately, the serum may be kept on ice and examined within the following forty-eight hours.

A few points on the manipulation of the serum follow. The writer has found that his automatic pipet,<sup>12</sup> recently described, measures fluids better than can be done by hand, saving much time and insuring accuracy after its simple operation has been learned. In estimating nonproteins 0.5 c.c. of serum has been found sufficient for each test. To assure accuracy, two controls for globulin estimations and one control for nonprotein estimations are advised, thus making the setting

TABLE 3.—EFFECT OF HIGH PROTEIN DIET ON SERUM PROTEINS

Case No.	Diagnosis	Low Protein Diet for Four Days; Serum Proteins				Average Daily Urinary N Output in Grams*	High Protein Diet for Four Days; Serum Proteins				Average Daily Urinary N Output in Grams*	Variations in Serum Proteins Due to High Protein Diet			
		Albu- min	Glob- ulin	Total Pro- tein	Non- pro- tein		Albu- min	Glob- ulin	Total Pro- tein	Non- pro- tein		Albu- min	Glob- ulin	Total Pro- tein	Non- pro- tein
1	Fractured femur	4.5	2.9	7.4	1.2	6.6	4.7	2.7	7.4	1.3	15.6	0.2	-0.2	0	0.1
2	Fractured femur	5.2	1.9	7.1	1.3	....	5.5	2.3	7.8	1.3	....	0.3	0.4	0.7	0
3	Septic knee	3.8	3.4	7.2	1.5	6.66	4.3	3.4	7.7	1.3	12.3	0.5	0	0.5	-0.2
4	Fractured tibia	5.8	2.2	8	1.1	5.95	5.6	2.3	7.9	1.25	20.16	-0.2	0.1	-0.1	0.15
5	Fractured hip	5.1	2.2	7.3	1.2	5.79	5.1	2.3	7.4	1.3	11.7	0	0.1	0.1	0.1
6	Septic compound fract.	4.4	2.7	7.1	1.3	4.4	4.7	2.6	7.3	1.3	9.27	0.3	-0.1	0.2	0

\* Estimation of urinary nitrogen was kindly furnished by Dr. W. Dennis.

up of five separate tubes for each serum. It has been found important to keep all tubes capped at all times after the fluid has been introduced, to prevent evaporation, this closure being done as advised by Robertson. Instead of platinum or silver wire, a rather thick chromium wire cut into lengths of about 1 or 1.5 cm. have been very satisfactory to bring about mixture of serum, sulphate solution and acetic acid solution. The separation of the coagulum resulting from boiling the nonprotein tubes is accomplished by means of a slender glass rod rather than by a platinum wire. A piece of chromium wire would serve the same purpose. In order to prevent bumping during the boiling of these nonprotein tubes, a double piece of wire gauze cut to fit the bottom of the beaker is better than cotton. The refractometric

12. Rowe: Jour. Lab. and Clin. Med., 1916, 1, 439.



readings have been made in a fairly dark room where, by means of a window and door, the temperature was kept nearly constant during the readings.

That this temperature regulation serves the same purpose as does the complicated Pulfrich temperature regulating apparatus is shown by the following results obtained by examining a serum by the University of California instrument, which we used, and by a refractometer

TABLE 4.—CHANGES IN SERUM PROTEINS CAUSED BY KEEPING SERUM ON ICE

Case No.	Serum Proteins within 4 Hours after Blood-Drawing					Time Serum Stood on Ice between Examinations, Hours	Variations in Serum Proteins after Serum Stood on Ice				
	Albu- min	Glob- ulin	Total Pro- tein	Non- pro- tein	Per Cent. of Glob- ulin		Albu- min	Glob- ulin	Total Pro- tein	Non- pro- tein	Per Cent. of Glob- ulin
1	4.2	2.3	6.5	1.3	36	48	0	-0.1	-0.1	0	-2
2	4.8	3.1	7.9	1.2	40	48	0.1	-0.3	-0.2	0.1	-4
3	5.1	2.9	8	1.6	36	24	0	0	0	0	0
4	5.7	2	7.7	1.1	26	24	0.3	-0.1	0.2	-0.1	-1
5	5.8	2.9	8.7	1.3	33	24	-0.3	0.2	-0.1	0.1	3
6	4.3	2.1	6.4	1.4	33	24	0.2	-0.1	0.1	0	-2
7	4.9	2.2	7.1	1.1	31	24	-0.2	0	-0.2	0.2	1
8	4.8	2.1	6.9	1.3	30	24	-0.1	0	-0.1	0	1
9	5.9	3	8.9	1.3	34	24	-0.1	0.1	0	0.1	1
10	4.4	2.2	6.6	1.5	33	24	0.1	-0.3	-0.2	0	-3
11	4.5	2	6.5	1.4	31	24	0.2	-0.2	0	0	-3

belonging to the Institute of Technology, which had the temperature regulating device:

	Univ. of Calif., Per Cent.	Technology, Per Cent.
Albumin .....	5.2	5.24
Globulin .....	2.07	2.09
Total protein .....	7.27	7.33
Nonprotein .....	1.3	1.3

The agreement of these results shows the accuracy of the instrument used in our investigations and also the possibility of obtaining accurate results by keeping the room temperature constant. In order that the fluids to be examined might be at room temperature they were left in that room one-half hour before readings were taken. If a 15 watt electric light bulb is suspended just above and in front of the refractometer, the readings may be made without turning the light on and off.

POSSIBLE CLINICAL USE OF THIS METHOD

For clinical use this method of investigation of blood serum is practical, especially if in diseases in which no definite increase in nonproteins has been found, our average value of 0.00198 for these nonproteins is used. As stated in a previous article,<sup>13</sup> if this fixed value is used, the serum examination is greatly shortened and an unreasonable error is not introduced. In severe nephritis, especially where uremia<sup>14</sup> is present, it is necessary to make determinations of nonproteins in each serum to obtain accurate results.

We have shown that during certain diseased conditions, especially generalized infections and cardiorenal diseases, that the total proteins may be decreased and the percentage of globulin in the total proteins increased, and that recovery is accompanied by a return of these values to normal levels. A guide of some worth as to prognosis and treatment seems to reside in the following of these values during the course of the disease, especially in chronic nephritis with edema. Diabetes has been found to be accompanied by normal percentages of

TABLE 5.—EFFECT OF HEMOLYSIS ON SERUM PROTEINS

Case No.	Serum Proteins before Hemolysis					Increase in Serum Proteins Due to Hemolysis				
	Albu- min	Glob- ulin	Total Pro-	Nonpro- tein	Per Cent. of Glob- ulin	Albu- min	Glob- ulin	Total Pro-	Nonpro- tein	Per Cent. of Globulin
1	5.7	2	7.7	1.3	26	0.2	0.2	0.4	0.1	1
2	5.9	1.6	7.5	1.3	21	0.5	0.2	0.7	0.05	1

globulin, except for an increase when a complicating infection is present. Other points of clinical interest are mentioned throughout our other articles.

We have commented before on the inaccuracy of estimating total proteins by the table of Reiss.<sup>15</sup> This error would be slightly less if 0.00198 were substituted for his value of 0.00277 for nonproteins. Determination of total proteins, though, by this method should be used only when a rough idea of the serum concentration is desired, in such cases as Reiss mentions in his last article, for instance, in which a daily comparison of serum concentration with body weight would be of use.

SUMMARY

Muscular activity, even of the simplest sort, increases total serum proteins, this increase occurring more in the albumin than the globulin fraction.

13. Rowe: THE ARCHIVES INT. MED., 1916, **18**, 455.

14. Rowe: THE ARCHIVES INT. MED., 1917, **19**, 354.

15. Reiss: Deutsch. Arch. f. klin. Med., 1915, cvii, 175.

An immediate effect of diet on serum proteins is practically wanting, while a high protein diet continued for several days produces a slight increase in the serum proteins.

The percentage of globulin seems to be slightly increased in cases in which healing of fractures or operative incisions is occurring and in which no signs of infection are present.

Serum proteins undergo no marked changes after the serum has been kept on ice for forty-eight hours.

Hemolysis, when excessive, definitely increases serum concentration, while slight hemolysis produces only a very slight increase.

A few suggestions about the technic and clinical use of Robertson's microrefractometric method are given.



# THE CAUSES OF VARIATION IN THE CONCENTRATION OF UREA IN THE BLOOD OF YOUNG HEALTHY ADULTS \*

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It is known that there may be a variation of more than 100 per cent. in the blood urea concentration of any group of normal subjects. It is not known why this considerable degree of variation should exist. From the available data it is possible to suppose that each person has his own characteristic blood urea concentration, which varies only within narrow limits, although the variation between different subjects is so large. Or it may be that the blood urea concentration in each person varies widely in accordance with one or other of the many constantly changing conditions of the body.

One cause of variation in the concentration of some constituents of the blood of different persons is a variable food intake. And before any study of the effect of physiologic variables on the blood urea concentration can be carried out or any attempt made to draw any but the broadest line between normal and abnormal, it is necessary to determine the range of variation in subjects in whom this possible cause of variation has been removed by the imposition of a qualitatively and quantitatively constant diet. Yet we do not so much as know whether food alterations can influence the blood urea concentration in normal subjects. It is only in the most recent work that even inadequate attention has been paid to the possibility of the effect of dietary factors. Bang<sup>1</sup> carried out determinations of nonprotein nitrogen, urea nitrogen and amino-acid nitrogen on eight students while they were on a diet which was more or less qualitatively the same, though it is not stated that any quantitative restrictions were imposed. His methods are analogous to his micromethod for sugar estimation in the blood, in that only one or two drops of blood are used. He found a variation of from 0.006 to 0.022 per cent. urea nitrogen before breakfast. He states that under ordinary conditions dietary changes have no effect on the blood urea concentration of normal persons. Schwartz and McGill<sup>2</sup> found a variation of from 0.0108 to 0.0156 per cent. urea nitrogen in four normal subjects after

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\* From the Laboratory of the Medical Division of the Leland Stanford Junior University School of Medicine, San Francisco.

1. Bang, I.: *Biochem. Ztschr.*, 1915, **62**, 104.

2. Schwartz, H., and McGill, C.: *THE ARCHIVES INT. MED.*, 1916, **17**, 42.

TABLE 1.—THE EFFECT OF VARIATION IN DIET ON THE UREA CONCENTRATION OF THE BLOOD IN NORMAL INDIVIDUALS

Date	Diet			Urea in Urine 24 Hours, Gm.	Urea in Blood, Grams per 100 C.c.
	Protein, Gm.	Carbohy- drate, Gm.	Fat, Gm.		
Subject Add.					
4/17-18	12	335	45	16.62	
4/18-19	12	333	45	10.92	
4/19-20	12	390	45	9.2	
4/20-21	12	328	45	8.39	0.0225
4/21-22	12	450	45	8	0.027
4/22-23	12	390	45	8.25	
4/23-24	152	7	212	15.81	
4/24-25	145	6	218	28.29	
4/25-26	130	6	229	36.1	
4/26-27	148	12	284	36.09	0.0567
4/27-28	115	8	235	34.24	0.0469
4/28-29	110	6	225	32.7	0.0426
4/29-30	75	220	120	21.95	
4/30- 1	75	220	120	14.02	
5/ 1- 2	75	220	120	15.82	
Subject Mo.					
4/17-18	12	342	45	.....	
4/18-19	12	382	45	12.32	
4/19-20	12	390	45	10.14	
4/20-21	12	491	45	8.74	0.024
4/21-22	12	497	45	10.55	0.0252
4/21-23	12	440	45	6.25	0.0252
4/23-24	155	9	209	18.49	
4/24-25	152	7	249	32.6	
4/25-26	164	9	309	47.28	
4/26-27	192	5	279	41.95	0.0468
4/27-28	149	9	255	50.02	0.0369
4/28-29	215	3	279	49.45	0.0402
4/29-30	75	220	120	29.20	
4/30-31	75	220	120	16.31	
5/ 1- 2	75	220	120	13.15	
Subject Bo.					
5/ 2- 3	11	402	76	16.22	
5/ 3- 4	11	482	76	11.88	
5/ 4- 5	11	490	76	7.17	
5/ 5- 6	11	455	76	7.36	0.0168
5/ 6- 7	11	455	76	8.34	0.0169
5/ 7- 8	11	455	76	5.74	0.0228

TABLE 1.—THE EFFECT OF VARIATION IN DIET ON THE UREA CONCENTRATION OF THE BLOOD IN NORMAL INDIVIDUALS—(Continued)

Date	Diet			Urea in Urine 24 Hours, Gm.	Urea in Blood, Grams per 100 C.c.
	Protein, Gm	Carbohy- drate, Gm	Fat, Gm.		
5/ 8- 9	161	4	238	25.08	
5/ 9-10	161	4	238	43.24	
5/10-11	161	4	238	40.35	
5/11-12	161	4	238	37.42	0.039
5/12-13	161	4	238	35.58	0.041
5/13-14	161	4	238	35.18	0.042
Subject H. 5/ 2- 3	11	437	76	13.02	
5/ 3- 4	11	432	76	8.26	
5/ 4- 5	11	407	76	6.01	
5/ 5- 6	11	417	76	5.57	0.0147
5/ 6- 7	11	412	76	6.13	0.0125
5/ 7- 8	11	417	76	5.36	0.0228
5/ 8- 9	154	4	233	21.87	
5/ 9-10	161	4	258	44.35	
5/10-11	154	4	233	49.5	
5/11-12	154	4	233	42.23	0.0438
5/12-13	134	4	177	40.05	0.0357
5/13-14	154	4	233	44.24	0.0384
5/14-15	75	220	120	26.62	
5/15-16	75	220	120	16.55	
5/16-17	75	220	120	14.4	

seventeen hours' fasting. In fifteen normals two and a half hours after "a heavy protein meal" the variation was from 0.0108 to 0.0252 per cent. urea nitrogen. Gettler and Baker<sup>3</sup> have published a very valuable series of determinations of various blood constituents in thirty normal persons. They found a variation of from 0.01 to 0.026 per cent. urea nitrogen three hours after a standard breakfast.

It does not appear certain from these data that dietary changes have any effect on the urea concentration of the blood of normal subjects, although Folin, Denis and Seymour<sup>4</sup> have shown that in patients with high blood pressure an increase of protein in the diet leads to an increase in the urea concentration of the blood. This, however, might be and no doubt has been ascribed entirely to defective elimina-

3. Gettler and Baker: Jour. Biol. Chem., 1916, **25**, 211.

4. Folin, O., Denis, W., and Seymour, M.: THE ARCHIVES INT. MED., 1914, **13**, 224.



tion of urea by the kidneys. With kidneys of unimpaired efficiency, on the other hand, it might well be imagined that any marked alteration in the level of urea in the blood would be obviated by an automatic increase in the rate of urea excretion whenever the urea concentration of the blood began to rise. But this is not what happens. In healthy subjects as well as in those whose kidneys may be defective, an increased nitrogen consumption is accompanied by an increased urea content of the blood. This is demonstrated in the experiments detailed in Table 1.

It will be noted that there is a considerable increase in blood urea concentration<sup>5</sup> when the food is changed from a mainly carbohydrate to a protein-fat diet. Therefore, in order to determine what degree of variation may arise from other than food factors, it is necessary that all the subjects should be on the same diet.

The figures given in Table 2 were obtained at 10:30 a. m. on the third and sixth days of the same diet. On the fourth, fifth and sixth days of the diet 20 or 40 gm. of urea were taken at 8 a. m. The subjects were instructors and students between the ages of 20 and 35 years. The diet has been given in detail elsewhere,<sup>6</sup> as well as the method used for the estimation of urea (a slight modification of Van Slyke and Cullen's method).<sup>7</sup> Five c.c. of blood was the smallest amount used for each estimation. Duplicate estimations were made in the great majority of cases.

The chief interest of these figures seems to us to lie in their demonstration of the diversity of concentration which may exist in normal subjects in spite of uniformity of diet. This is particularly true of the third day of the diet, for after urea administration the range of variation becomes relatively narrower. But before urea was taken we have variations of from 0.0156 to 0.0438 per cent.

Eighty-two per cent. of our twenty-eight cases had a concentration of 0.02 per cent. Only three cases, Ad., We. and M., show a wide variation from the average. It will be noted that these three cases three days later after urea was taken still show an unusually high or low urea concentration. It would appear then that these differences are to be ascribed in part at least to individual peculiarities which continued to exist throughout the period of the test and are not due altogether to temporary and evanescent causes of variation. Now we have shown that the rate of excretion of administered urea

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5. All the figures for blood urea concentration in this paper refer to grams of urea per hundred c.c. of blood and not to urea nitrogen. The values for urea nitrogen may be approximately obtained by dividing our results by 2.

6. Addis, T., and Watanabe, C. K.: *Jour. Biol. Chem.*, 1916, **27**, 249. There was no egg given with the noonday meal, as is incorrectly stated in this paper.

7. Addis, T., and Watanabe, C. K.: *Jour. Biol. Chem.*, 1916, **24**, 203.

TABLE 2.—CONCENTRATION OF UREA IN THE BLOOD OF NORMAL INDIVIDUALS ON A CONSTANT DIET ARRANGED IN ORDER OF DESCENDING MAGNITUDE

Blood Urea Concentration, Gm. per 100 C.e.					
Subject	Before Urea Administration, 3d Day 10-11 a. m.	Subject	After 20 Gm. Urea at 8 a. m., 6th Day 10-11 a. m.	Subject	After 40 Gm. Urea at 8 a. m., 6th Day 10-11 a. m.
Ad.	0.0438	Ad.	0.075	*Eh.	0.123
D.	0.0312	A.	0.069	*Sh. <sub>2</sub>	0.1125
Bo. <sub>2</sub>	0.03			*K. <sub>2</sub>	0.105
Sh. <sub>1</sub>	0.0292	Add.	0.0678	*Bo. <sub>2</sub>	0.098
G.	0.0288	Bo. <sub>1</sub>	0.0672	*T.	0.09
A.	0.0282	K. <sub>2</sub>	0.0672		
Add.	0.0276	K. <sub>1</sub>	0.066		
*Sh. <sub>2</sub>	0.027	D.	0.0651		
Bo. <sub>1</sub>	0.0261	J.	0.063		
Br. <sub>1</sub>	0.0252	Wa.	0.063		
Cha.	0.0252	C.	0.0624		
T.	0.0252	G.	0.0612		
J.	0.0252	S. <sub>1</sub>	0.0606		
Wa.	0.025	Br. <sub>1</sub>	0.06		
C.	0.0246	Boy	0.059		
*McC.	0.0246	C.	0.0558		
W.	0.0246	F.	0.0558		
K. <sub>1</sub>	0.0246	M.	0.0558		
		O.	0.0546		
B.	0.024	B.	0.0516		
F.	0.024	We.	0.051		
S. <sub>1</sub>	0.024	W.	0.0492		
*K. <sub>2</sub>	0.0216				
*K. <sub>3</sub>	0.0216				
C.	0.021				
W.	0.021				
Ma.	0.021				
O.	0.0204				
We.	0.018				
M.	0.0156				

\* Blood collected at 11 a. m. instead of 10:30 a. m. The inferior figures indicate repetitions of the diet by the same individual.

in normal persons is remarkably constant and that these three subjects were not exceptions to this rule.<sup>6</sup> The high blood urea concentration of Ad. is therefore certainly not due to a relative insufficiency of his kidneys, nor did the low concentrations in We. and M. arise because their kidneys were exceptionally efficient. We believe that the explanation is to be found in the fact that the level of protein catabolism

was high in the subject Ad. and low in We. and M. Although these subjects took the same amount of nitrogen, the urea excretion over the whole period of the diet, with the exception of the first day, was 142.9 gm. for Ad. and only 123.8 gm. for We. and 110.4 gm. for M. The high urea excretion of Ad. presumably arose from an increased conversion of amino-acids from his tissues and from the protein of his food into urea, whereas the low urea excretions of We. and M. may be accounted for by a decreased tissue catabolism and a high degree of utilization of food amino-acids for protein synthesis, with a consequent decrease in urea formation.

In explanation of this effect of increased protein catabolism in raising the blood urea concentration, it is not necessary to assume any peculiarity in the urea derived from tissue catabolism whereby it is more difficult to excrete and so tends to heap itself up in the blood. For, as is shown by our results after urea administration, exactly the same effect is produced by preformed urea taken by mouth. Urea, whether from the alimentary tract or from the tissues, will tend to raise the blood urea concentration whenever it enters the blood more rapidly than it leaves it through the kidneys. The kidneys are not so constituted as to be close regulators of the level of urea in the blood, in the manner, for instance, in which the respiratory center maintains an even hydrogen ion concentration. There is no necessity for any such fine adjustment, since the properties of urea are such that considerable fluctuations in concentration are immaterial to the body.

But we believe that alterations in blood concentration may occur also as a result of changes in the sensitiveness of the kidneys to the stimulation to an increased rate of urea excretion which is provided by rises in blood urea concentration. Under the constancy of the conditions imposed on the series of persons we have detailed above, this factor was probably of very minor importance, yet even there it seems probable that the rate of protein catabolism does not explain all the variation found, for there are instances in which there is no very close correspondence between the blood urea concentration and the rate of protein catabolism. But it is particularly under widely different conditions that this factor of varying states of kidney activity seems to become evident. Thus, in a case of diabetes, after forty-eight hours during which nothing was taken but whisky and water, we gave 20 gm. of nitrogen-free corn starch, which did not induce any glycosuria. Some hours later we estimated the blood urea concentration in the expectation that we would find an unusually low concentration, because the rate of protein metabolism, as estimated by the urinary excretion, was low, the carbohydrate had been utilized and all exogenous sources of urea cut off. Yet 0.063 per cent. was found. A month later, after the patient had been for some time on a



diet with a moderate amount of protein and almost no carbohydrate we found a concentration of only 0.0462 per cent. a few hours after a breakfast of two eggs and some bacon. The rate of urea excretion in the urine on the first occasion was only 0.48 gm. per hour, whereas at the second examination, in spite of the lower blood concentration, it was 1.43 gm. per hour. This is only the most marked of a series of observations which have led us to conclude that the level of urea in the blood may increase, not only because urea comes in too quickly, but also because under certain conditions it may leave the blood too slowly. In other words, there is no necessarily fixed relationship between the concentration of urea in the blood and the rate of urea excretion, since other factors than urea play a part in determining the rate of excretion.

The wide range of variation in these results is also of interest in connection with the significance to be attached to estimations of blood urea concentration in patients. On a diet such as ours, a conservative estimate would call abnormal only concentrations which did not fall within from 0.01 to 0.05 per cent. But when, as must commonly be the case, the patient has not been subjected to any dietetic preparation, figures beyond these limits may still possibly be normal, since under variable food conditions the normal range of variation will be widened.

The simplest way to attain some degree of constancy is to take the blood in the morning before breakfast. It is, however, not to be expected that in this manner any such uniformity can be attained as is possible when a constant diet is taken for several days. When a person passes from an unrestricted diet containing a moderate quantity of nitrogen to a fixed diet containing slightly less nitrogenous food, it requires on an average a full twenty-four hours for an equilibrium in the excretion of urea to be established. But when the nitrogen in the food taken before the diet exceeds at all markedly the amount in the fixed diet, the excretion of the excess urea is apparent for as long as forty-eight hours. Constancy as regards diet, therefore, is not to be attained by abstention from food for the twelve to seventeen hours, which represents the time between the evening meal on the previous day and the taking of the blood before breakfast. Yet in clinical work this is not infrequently the highest degree of uniformity in conditions which can be obtained.

We have thought it well, therefore, to collect all our observations on normal subjects when the blood was taken before food on a day following unrestricted diet. Some of these subjects had taken water, but as we shall show later, this does not demonstrably alter the blood urea concentration. In order that the extent of the error inherent in the method of estimation may be appreciated, we have given both the results obtained in all those cases in which double estimations were

TABLE 3.—CONCENTRATION OF UREA IN THE BLOOD OF NORMAL FASTING ADULTS

ARRANGED IN ORDER OF ACENDING MAGNITUDE; DUPLICATE ESTIMATIONS

No.	Subject	Blood Concentration in 100 C.c.		No.	Subject	Blood Concentration in 100 C.c.	
1	V.	0.0225		40	Jo.	0.0336	0.0336
2	Add.	0.0246	0.0246	41	Add.	0.0337	
3	Add.	0.0264	0.0264	42	So.	0.0342	0.033
4	Add.	0.0264	0.0264	43	Add.	0.0342	0.033
5	Add.	0.027	0.0258	44	Ka.	0.0337	
6	Mon.	0.027	0.0264	45	Add.	0.0354	0.0348
7	V.	0.027		46	Add.	0.0354	0.0354
8	Add.	0.0276	0.0276	47	Add.	0.036	0.0354
9	Mon.	0.0282	0.027	48	Ka.	0.036	
10	Mon.	0.0282		49	Add.	0.036	0.036
11	Kl.	0.0282	0.0282	50	Mon.	0.036	0.036
12	Mon.	0.0288	0.0276	51	Wl.	0.0366	0.036
13	Cal.	0.029		52	So.	0.0366	0.036
14	Sh.	0.0292		53	Add.	0.0367	
15	Cal.	0.0292		54	Add.	0.0372	0.0364
16	Add.	0.0294	0.0294	55	Sha.	0.0379	
17	Mon.	0.03	0.0288	56	So.	0.0382	
18	Wl.	0.03	0.0294	57	Cal.	0.0384	0.0372
19	Add.	0.03	0.0294	58	Ja.	0.0384	0.0378
20	Add.	0.03		59	Mo.	0.0384	0.0381
21	Add.	0.03	0.03	60	Ch.	0.039	0.039
22	Add.	0.03	0.03	61	Ja.	0.039	
23	Add.	0.03		62	Ja.	0.0397	
24	Ka.	0.0307		63	Ja.	0.0398	0.0392
25	Add.	0.0312	0.0306	64	Add.	0.0402	0.039
26	Add.	0.0312	0.0312	65	Mo.	0.0402	0.0386
27	Add.	0.0312	0.0312	66	Ka.	0.0402	0.039
28	Mon.	0.0315	0.0315	67	Mo.	0.0402	
29	Add.	0.0315		68	Jo.	0.0405	
30	Add.	0.0318	0.0306	69	Add.	0.0408	0.039
31	Mon.	0.0318	0.0312	70	F.	0.0408	
32	Add.	0.0322		71	Add.	0.0414	0.0408
33	Add.	0.0322		72	Add.	0.0414	0.0414
34	Mon.	0.0324	0.0312	73	Add.	0.0414	0.0408
35	Boy	0.0324	0.0318	74	Pr.	0.042	0.0414
36	Sh.	0.033		75	Jo.	0.0426	0.042
37	Add.	0.0333	0.033	76	Add.	0.0426	0.0426
38	Add.	0.0336	0.0324	77	Add.	0.0428	0.0426
39	Ka.	0.0336	0.033	78	Add.	0.0432	0.0426

TABLE 3.—CONCENTRATION OF UREA IN THE BLOOD OF NORMAL FASTING ADULTS—(Continued)

ARRANGED IN ORDER OF ASCENDING MAGNITUDE; DUPLICATE ESTIMATIONS

No.	Subject	Blood Concentration in 100 C.c.		No.	Subject	Blood Concentration in 100 C.c.	
79	Mo.	0.0435		93	Boy	0.0468	0.0462
80	Sha.	0.0435		94	Mo.	0.0485	
81	Add.	0.0438	0.0426	95	Sh.	0.0488	0.0456
82	Mon.	0.0438		96	Add.	0.0489	
83	Cal.	0.045	0.0444	97	Add.	0.0498	0.0498
84	Cal.	0.045		98	Mo.	0.051	0.051
85	Add.	0.045		99	Mon.	0.051	0.051
86	Add.	0.045	0.0438	100	Add.	0.0516	0.0516
87	Add.	0.045	0.0444	101	Add.	0.0522	0.0504
88	Mo	0.0462	0.045	102	Add.	0.0528	0.0516
89	Add.	0.0462	0.0462	103	Sha.	0.0547	
90	Add.	0.0462	0.045	104	Add.	0.0564	0.0552
91	Add.	0.0462	0.045	105	Wy.	0.0598	
92	Add.	0.0485		106	Add.	0.06	0.0598

carried out. The results are arranged in order of ascending magnitude in Table 3.

The average of this series is 0.038 per cent., while the average of the series on the third day of the diet was 0.0251 per cent. The increase was to be expected, since the diet contained only 75 gm. of protein—somewhat less than the average protein intake of healthy subjects. The range of variation—from 0.0225 to 0.0600 per cent.—is also greater, as was to be expected from the greater variability of the conditions.

The most striking and interesting point in the table is the demonstration of the fact that the variation in one person is as great as the variation in the whole group. Fifty out of these 106 estimations were carried out on one subject (Add.). His blood concentration varied from 0.0246 to 0.06 per cent., which is practically the group variation. This was not the result of wide fluctuations in protein intake on the days before the blood was taken, for this subject habitually took a very moderate and not unusually inconstant amount of protein food. Nor is it an isolated instance of a special tendency toward inconstancy in the level of blood urea, for the subject Mon., on whom only eleven estimations were made, also shows the wide variation of from 0.027 to 0.051 per cent. This is a strong argument against the view that the variation in urea concentration in one subject as compared with another is likely in any of our cases to be



conditioned by any such permanent individual peculiarities as might be produced by structural differences in their kidneys. For if the same subject shows as great a variability as is manifested by the whole group, the cause of the group variability must be sought, not in any fixed differences in the kidneys, which regulate the blood concentration, but in an inconstancy of the conditions under which these kidneys were placed. We have shown that even when all dietary inconstancies are removed, there is still a high degree of variability. The variability in this series is therefore not wholly nor even mainly explained by differences in food intake. It is due, we think, for the most part to inconstancy in the rate of formation of urea in the processes of protein catabolism. During the two years during which these fifty observations were made on the subject Add. he may have passed through phases of decrease and increase in protein catabolism which might account for the wide variation in blood urea concentration which was found.

If changes in the rate of protein catabolism under physiologic conditions are accompanied by considerable fluctuations in the level of blood urea concentration, one would expect to find a still wider variation arising from the exaggerated disturbances in protein metabolism which occur in certain pathologic states. A marked increase in the concentration of nonprotein nitrogen or of urea in the blood has been noted in some diseases which are not necessarily associated with any renal deficiency. These include certain cases of pneumonia<sup>8</sup> and carcinoma,<sup>9</sup> as well as intestinal obstruction,<sup>10</sup> protease poisoning<sup>11</sup> and hyperthyroidism.<sup>12</sup> These are eminently conditions in which an increased protein catabolism may be expected.

In clinical work, blood urea estimations are at present usually carried out in order to determine whether or not there is an insufficiency in kidney function. It is obvious that the wide range of variation which exists precludes such determinations from being taken as an accurate measure of kidney function. Still the very highest levels of blood urea concentration are apparently only attainable when kidney elimination is defective. Concentrations above 0.15 per cent. speak decisively for renal decompensation. But below that figure judgment will be required in every case. A concentration of 0.1 per cent. may be very strong evidence of renal deficiency in one case, while in another in which there is reason to expect an increased rate of protein catabolism or in which unusual dietary or other conditions are present it may not justify more than a suspicion.

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8. Herter: Johns Hopkins Hosp. Rep., 1900, **9**, 69.

9. Strauss, quoted by Monakow, P.: *Deutsch. Arch. f. klin. Med.*, 1914, **115**, 47.

10. Tileston, W., and Comfort, C. W.: *THE ARCHIVES INT. MED.*, 1914, **14**, 620.

11. Whipple: *Jour. Am. Med. Assn.*, 1915, **65**, 476.

12. Schwartz, H., and McGill, C.: *THE ARCHIVES INT. MED.*, 1916, **17**, 42.

## CONCLUSIONS

1. Differences in diet are a cause of variation in the concentration of urea in the blood of normal persons.

A change from a mainly carbohydrate to a protein-fat diet was accompanied by an increase of from 58 to 250 per cent. in blood urea concentration.

2. On a constant diet a variation of from 0.0156 to 0.0438 gm. urea per 100 c.c. of blood was found in twenty-nine experiments on twenty-five normal persons.

3. Differences in the rate of protein catabolism are the principal cause of the variation which was found in the blood urea concentration of normal persons on a constant diet.

The subjects who had the greatest rate of protein catabolism had the highest blood urea concentrations, while in those subjects in whom protein catabolism was least the blood urea concentration was lowest.

4. The blood urea concentration of normal persons is not maintained at a constant level by a proportionate increase in the rate of excretion of urea from the blood by the kidneys, whenever there is an increase in the rate of entrance of urea from the tissues into the blood. Although under such circumstances an increase in the rate of urea excretion occurs, it is not sufficient to prevent some rise in the blood urea concentration. This rise takes place whether the increased rate of entry of urea from the tissues into the blood is produced by a greater formation of urea from protein taken as food or from the breaking down of tissue protein or from the absorption of preformed urea from the alimentary tract.

5. Under inconstant conditions variation in blood urea concentration may be caused by alterations in the activity of the kidneys.

The more constant the conditions, the more uniform is the action of the kidneys in responding to a rise in blood urea concentration by a definite though not directly proportional increase in the rate of urea excretion; and there is reason to believe that if all the conditions could be kept constant, no fluctuations in blood urea concentration would arise from any inconstancy in the function of the kidneys themselves. But under widely different conditions it can be seen that the kidneys, even of the same person, do not act in a uniform manner, so that the same rise in blood urea concentration may lead to a greater rate of excretion under certain conditions than it will under others.

6. Permanent individual peculiarities play no part as a cause of variation in the blood urea concentration of different normal persons.

In a group of twenty-two subjects a variation of from 0.0225 to 0.06 gm. urea per 100 c.c. of blood was found in a series of 106 estimations, carried out in the morning before breakfast. Practically the same degree of variation was shown by one of these subjects, on whom fifty estimations were made.

# AUTOTRANSPLANTATION AND HOMO- TRANSPLANTATION

OF THE THYROID GLAND USING THE THYROID CAPSULE AS THE SEAT  
OF TRANSPLANTATION \*

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Having under our care a large number of cretins in whom cessation of the thyroid feeding leads rapidly to a recurrence of myxedema with its train of symptoms, we were led to undertake the following experimental work on autotransplantation and homotransplantation of the thyroid gland in the hope of obtaining sufficient positive results to warrant clinical operative interference. The following experimental operations are presented as a preliminary report.

## AUTOTRANSPLANTATION

We have found that the autotransplants of the thyroid gland into the thyroid capsule was successful in all our cases, corroborating the work of other investigators in autotransplantation.

We have studied three such cases of multiple autotransplantation.

## HOMOTRANSPLANTATION

The homotransplantation of the thyroid gland in animals is of great importance, and yet it has been unsuccessful up to the present time. Success in this form of transplant work would be a great step in solving the question of homotransplantation in general. The tendency of nearly all the workers, especially Manley and Marine,<sup>1</sup> von Eiselberg,<sup>2</sup> Payr,<sup>3</sup> Hesselberg,<sup>4</sup> Salzer,<sup>5</sup> Sermann<sup>6</sup> and Enderlen,<sup>7</sup> has been to

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1. Manley, O. T., and Marine, David: The Transplantation of Ductless Glands, with Reference to Ferments and Function, *Jour. Am. Med. Assn.*, 1916, **67**, 260.

2. Von Eiselberg: Transplantation of Thyroid and Parathyroid Gland, *Arch. f. klin. Chir.*, 1914, **106**, 1.

3. Payr, E.: Thyroid Gland Transplantation, *Arch. f. klin. Chir.*, 1914, **106**, 16.

4. Hesselberg, Cora: A Comparison of Autoplastic and Homeoplastic Transplantation of Thyroid Tissue in the Guinea-Pig, *Jour. Exper. Med.*, 1915, No. 21, p. 164.

5. Salzer, H.: A Contribution to the Transplantation of the Thyroid Gland, *Arch. f. klin. Chir.*, 1909, **89**, 861.

6. Sermann, Chava: About a New Method of the Transplantation of the Thyroid Gland, *Deutsch. Ztschr. f. Chir.*, 1908, **96**, 440.

7. Enderlen: Experiments on the Transplantation of the Thyroid Gland in the Abdominal Cavity of Cats and Dogs, *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1898, **3**, 474.



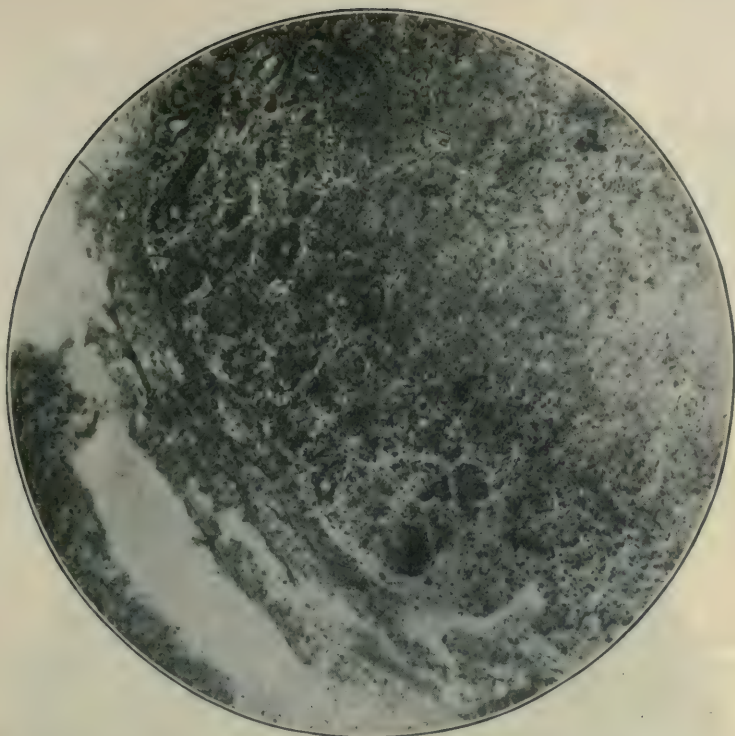


Fig. 1.—Autotransplant into loose areolar tissue in the neighborhood of the thyroid gland, twenty-five days after operation; magnification 15 diameters.

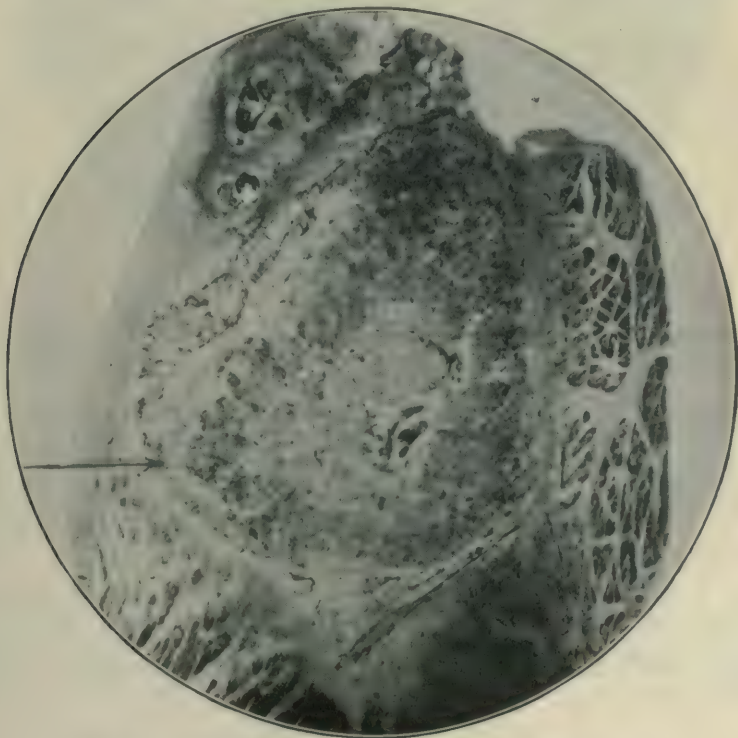


Fig. 2.—Same as Figure 1, magnification 60 diameters.

transplant the thyroid gland into some other portion of the body than into its normal location. Considering the highly specialized function of the organs of internal secretion; it seemed to us that the normal location was the best for definite functional reasons and therefore it seemed logical to us that we should attempt to transplant the organ in so far as possible at the seat of its normal blood supply.

We believe some of the important factors involved in a consideration of the normal position of this organ are, as follows:

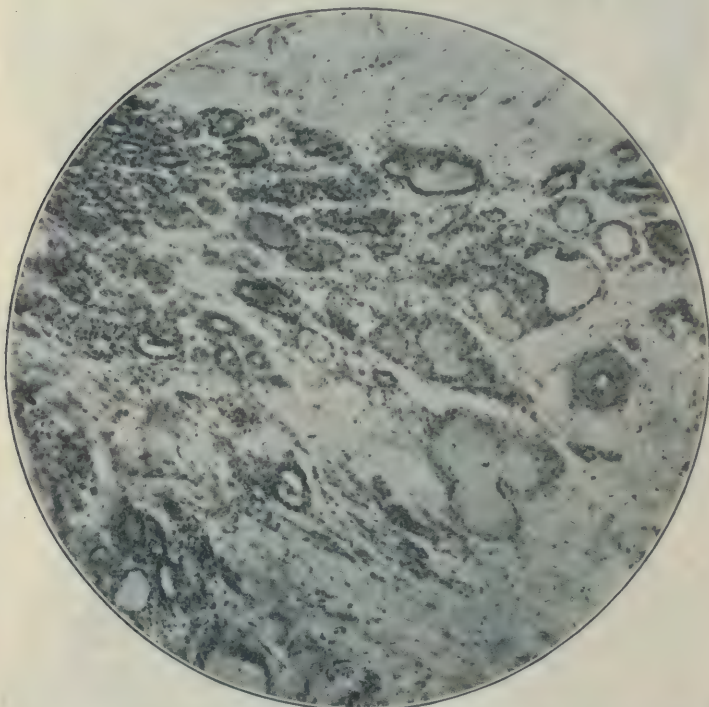


Fig. 3.—Autotransplant into capsule of thyroid gland, sixty days after operation; magnification 230 diameters.

1. The relation of the blood pressure and the size of the blood vessels nourishing this highly specialized organ must have an important bearing on the osmotic pressure of the blood and lymph as well as on the cellular structure of this particular organ, and consequently on its physiology.

2. It would appear that the chemistry of the tissue surrounding the thyroid transplant in intracapsular transplantation ought to be more adapted to its growth than would be the chemical reaction in foreign surroundings.

3. The secretion and iodine content of the remnants of the thyroid fragments within the capsule may be a factor in the retardation of the reaction between host and transplant in a manner similar to the results as shown by Marine, and Manley and Lenhart<sup>8</sup> in their iodine feeding of the host before the transplantation.

A considerable amount of experimental work has been done which, we believe, is conclusive proof that the quantity of secretion of the thyroid gland is regulated by stimuli reaching it through the circulation. Manley and Marine make the following statement in regard to their functional results in autotransplantation:

Inasmuch as such transplanted thyroid tissue undergoes all the morphologic variations associated with growth and function that are observed in nontransplanted thyroid tissue, and inasmuch as transplanted thyroid tissue shows the same reaction with iodine and the same storage of iodine as nontransplanted thyroid, we believe that this is sufficient evidence that such transplants may grow, involute or function equally as well as nontransplanted thyroid gland.

The above quoted experiments lead us to the same conclusions as these authors, to the effect that specific nerves, whether secretory or regulatory, are not necessary for normal growth or functional activity of the thyroid gland.

#### TECHNIC

The next important point in this series of transplants was the preservation of the normal blood supply of the thyroid gland of the host. We attempted to do all our transplant work without cutting off any portion of the blood supply to the gland. This was accomplished by placing our transplants into the thyroid capsule without ligation of any of the vessels supplying the thyroid gland. We accomplished this by splitting the thyroid capsule and removing part, or in some cases practically all of the thyroid tissue. During this time the assistant secured both poles of the thyroid gland between the thumb and index fingers, in this way controlling the hemorrhage without severely traumatizing the intima of the large blood vessels and at the same time establishing a soft thrombus. At the end of eight to ten minutes we found that there was practically no bleeding, and we were ready to insert our transplant into its new bed formed by the thyroid capsule. We believe that the avoiding of injury by clamps and ligatures applied to the vessels supplying the thyroid of the host is of great importance to the transplants. Necropsy showed a complete restoration of the circulation through these vessels in every case examined.

In almost all of the operations we avoided getting the sutures near

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8. Marine, David, and Lenhart, C. H.: On the Effects of the Administration or the Withholding of Iodine Containing Compounds in Normal, Colloid or Actively Hyperplastic Thyroids of Dogs, *THE ARCHIVES INT. MED.*, 1909, **3**, 66; *ibid.*, **4**, 253; *ibid.*, **4**, 440.



the transplanted portion in our closure of the capsule, for it is well known that any foreign body will cause a local leukocytic reaction and connective tissue hyperplasia about such a foreign body; therefore, our sutures were placed either in the capsule or the surrounding connective tissue as far from the transplant as was possible.

We used dogs and guinea-pigs as experimental animals, and performed three autotransplants and thirty-one homotransplants, twenty-seven of these being done on dogs and four on guinea-pigs, applying the above technic in the hope of producing successful results.

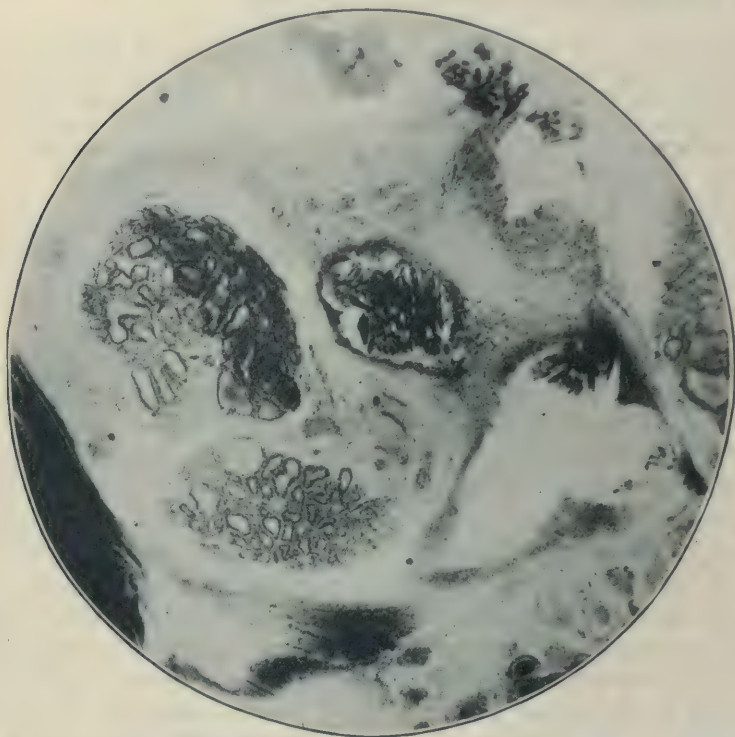


Fig. 4.—Homotransplant in puppy 6 weeks old, necropsy 120 days after operation; magnification 60 diameters.

After doing three autotransplants and fourteen homotransplants, using the foregoing operative technic but without paying any attention to the age and familial relationship of the experimental animals or to the type of gland as seen in the individual animals (and it should be remembered that the thyroids of dogs are subject to great histologic variation), we found that the homotransplants had practically disappeared within twenty to thirty days, with the exception of two cases, in which small islands of thyroid tissue remained as isolated areas surrounded by connective tissue. These latter were not of such size as

to offer any considerable encouragement as to the feasibility in human subjects.

We can corroborate the work of Cora Hesselberg, who describes these homotransplants as a degeneration and necrosis in the central portion of the transplant, with an infiltration of leukocytes, lymphocytes and connective tissue cells, which takes place in a varying degree in various animals, depending on the reaction that exists between the host and the tissue used for transplantation.

Believing that age, familial and histologic variations might be important factors in accounting for the failures with the technic given

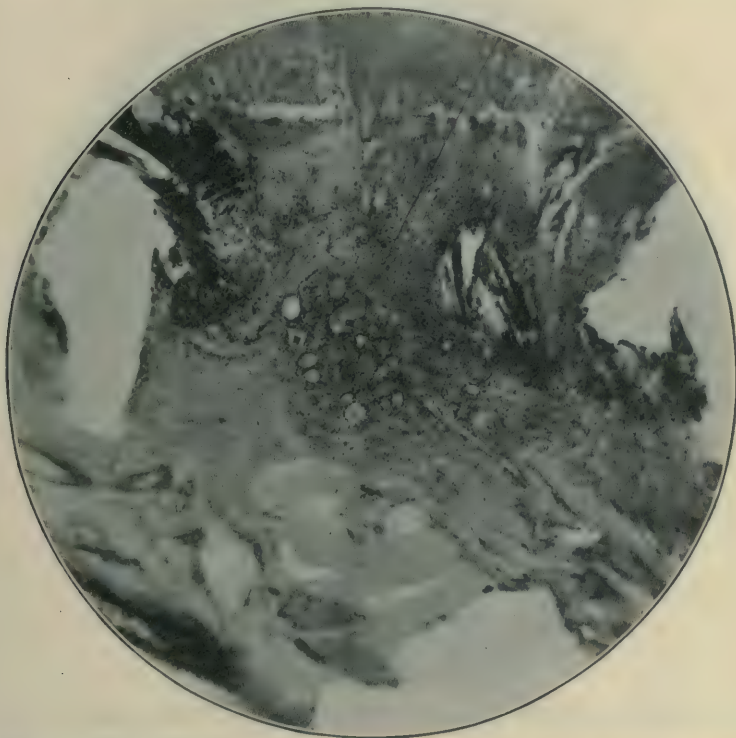


Fig. 5.—Homotransplant in puppy 7 weeks old, necropsy seventh day following operation; magnification 45 diameters.

above, we resorted to the use of young puppies of the same litter, aged from 2 to 6 weeks. We operated on eight puppies, using in each case those from the same litter. Seven of these puppies survived and were necropsied 7, 8, 19, 25, 60, 60 and 120 days, respectively, after the operation. In four of these we obtained living homotransplants, which can be described as follows:

There were small, isolated areas of thyroid alveoli, surrounded by new connective tissue or the capsule of the thyroid gland.

Many of the alveoli were seemingly normal in size. The cells were high and cuboidal in shape. Their nuclei stained well and the arrangement of the alveoli was normal. Many of the alveoli were filled with colloid material.

Dog 23 (Fig. 4), a female puppy, which was 6 weeks old when operated on, nursed its mother for several days following the operation and necropsy was performed 120 days after the operation. The transplant in this case was taken from a male puppy of the same litter. The surviving islands of thyroid tissue were embedded in areolar connective

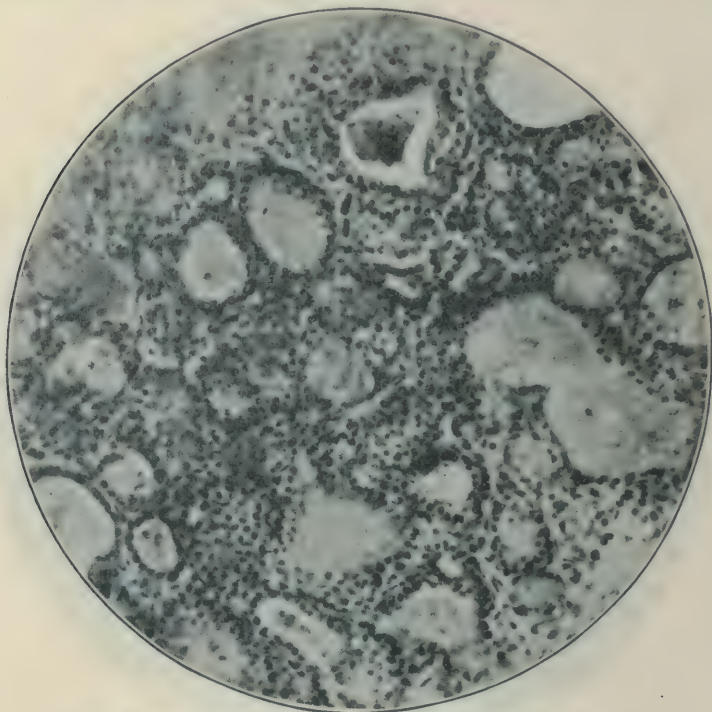


Fig. 6.—Same as Figure 5, magnification 230 diameters.

tissue, which was well organized with the remainder of the thyroid and connective tissue of the capsule proper.

Dog 24, a puppy, aged 7 weeks, was brought to necropsy on the seventh day following operation. The transplant had been taken from another puppy of the same litter (Figs. 5 and 6). There was a large area of transplant which showed quite marked degenerative changes and invasion of leukocytes and lymphocytes. At one side of this transplant there seemed to be a well-defined area of fairly normal-appearing thyroid tissue, which had the typical form of alveoli, filled with colloid material. The cells themselves were somewhat flattened, but the nuclei



stained very clearly and distinctly. This transplant showed changes similar to those seen in Dog 25, with the exception that there was not so much living thyroid in evidence.

Dog 25 (Figs. 7 and 8), a puppy, aged 7 weeks, of the same litter as Dog 24, from which the transplants were taken, was brought to necropsy on the eighth day. The transplanted thyroid showed a considerable hemorrhage, but not a great deal of infiltration of leukocytes and lymphocytes. There was a large part of this transplant which

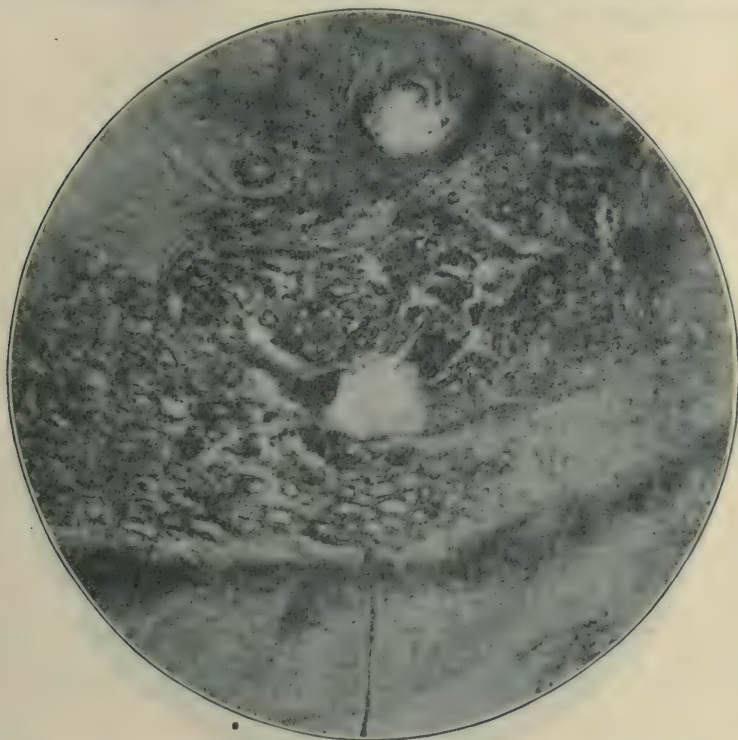


Fig. 7.—Homotransplant in puppy 7 weeks old, necropsy eight days after operation; magnification 50 diameters.

appeared almost normal. The alveoli of the living tissue were well arranged. Some of these alveoli were filled with colloid material. The nuclei stained well, but the cells were slightly flattened. Around the periphery of this transplant there was a large infiltration of leukocytes and lymphocytes. We believe that this thyroid transplant was living and well organized, but showed degenerative changes which were marked by the small alveoli and the flattening of the cells.

Dog 18, a puppy, aged 4 weeks (Fig. 9), was brought to necropsy sixty days after operation. A pocket had been made in the gland with-

out removing any of the capsule, and a piece of thyroid 1 by 2 by 4 mm. from a pup of the same litter had been planted into the capsule, which had been closed without suturing through the transplant. At necropsy the transplant showed some degenerative changes, some hemorrhagic areas, and some lymphoid and leukocytic infiltration, but there were many small, isolated areas of thyroid tissue, which, however, had lost their alveolar arrangement. There were a few alveoli present in this mass which showed a small amount of colloid. The thyroid cells in this mass stained perfectly and the nuclei were very distinct. This area lay just beneath the suture line.

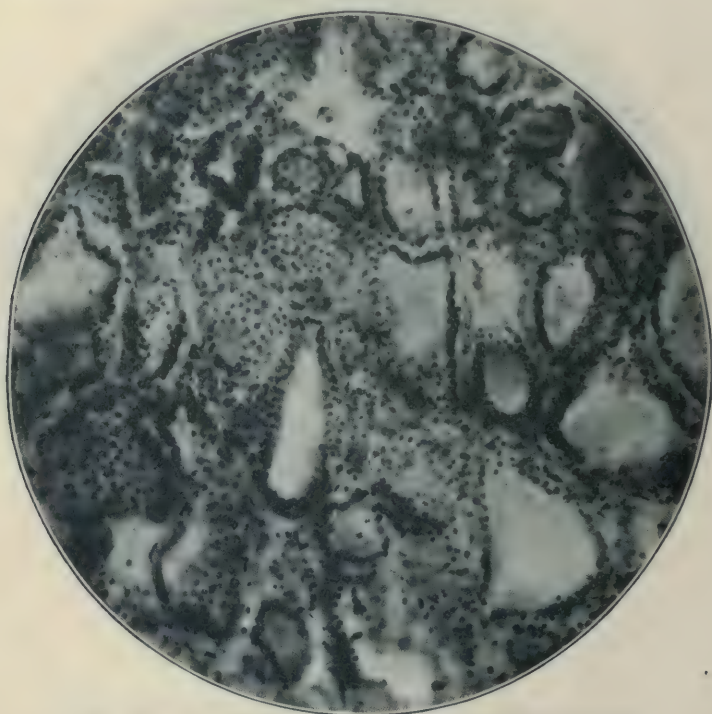


Fig. 8.—Same as Figure 7, magnification 230 diameters.

In the other three no such positive result was demonstrable. In two of these dogs, Puppies 16 and 17, the transplant was destroyed in sectioning the tissues.

In the four puppies described which were operated on with tissues taken from puppies of the same litter there seemed to be less destructive reaction between the host and the tissue transplanted into the thyroid gland than in the older dogs from different litters, and therefore these transplants survived in a manner similar to that seen in auto-transplantation, but with much smaller islands of living tissue. The

results shown in Figs. 5, 6, 7 and 8 may be open to question because of the short time between operation and necropsy, seven and eight days, respectively. However, we have based our conclusions on the well-preserved sections of tissues as shown in photomicrographs.

#### TRANSPLANTATION AFTER REPEATED PRELIMINARY TRANSFUSION

We cross-transfused several animals over a period of several weeks, giving from two to four injections at intervals of one week, using from 40 to 100 c.c. of freshly drawn citrated blood from each animal, which

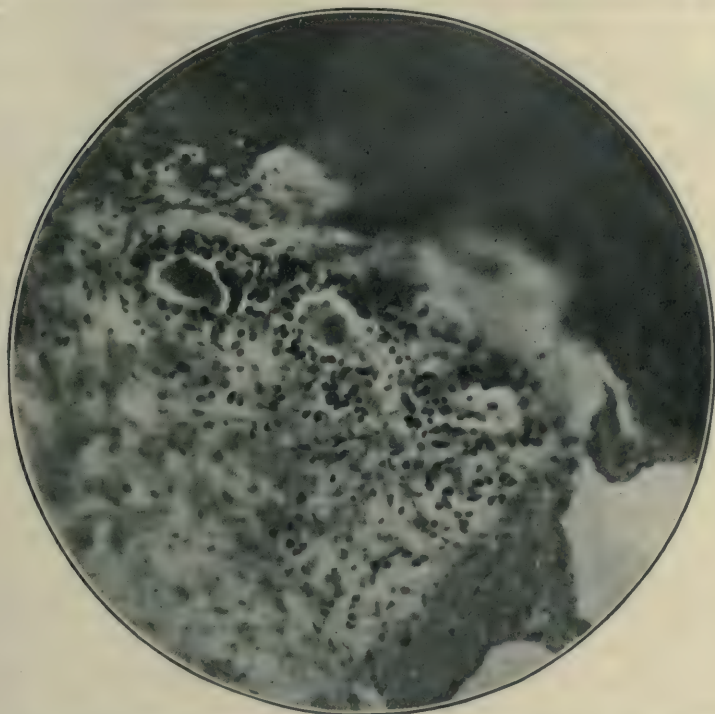


Fig. 9.—Homotransplant in puppy 4 weeks old, necropsy sixty days after operation; magnification 230 diameters.

was rapidly injected intravenously. Following this preparation, we cross-transplanted the thyroid glands, in each instance using the thyroid capsule as the seat of the transplant. Tests were made for the production of isohemolysins and iso-agglutinins in the two sets of dogs operated on, following two preparatory transfusions in one set and three in another set of dogs, but none were found.

We obtained the same results as Lexer and Morris, namely, this seemed to increase the reaction between the host and the transplanted tissue and thereby hastened the destruction of our transplants. It seems



to us, therefore, that to prepare the animal for transplantation would mean to prepare the blood and tissues in such a way that a leukopenia rather than a leukocytosis would be produced in the region of the transplant. To produce this seems to be the stumbling block in the efficiency of the homotransplant work.

#### CONCLUSIONS

We believe that the transplantation of autotransplants and homotransplants of highly specialized organs into the region normally occupied by these tissues is worthy of further trial.

We believe there should be a minimum disturbance of the blood supply in the region in which the transplant is placed, and that foreign bodies, such as suture material, should not come into contact with the transplant.

The varying degree to which a homotransplant takes depends on the amount of reaction between the host and the tissue transplanted, and so far we have found no means either in the blood or in the thyroid gland itself by which we could determine the factor which produces this different condition in the various animals.

A familial relationship and probably the early age of the animals on which operation was performed were important factors in our results.

We desire to thank Dr. J. J. Moore of the University of Illinois College of Medicine for the serologic work embodied in this article.

## THE EFFECT OF SALICYLATES ON EXPERIMENTAL ARTHRITIS IN RABBITS\*

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AND

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CHICAGO

In a study of this question by Dr. D. J. Davis,<sup>1</sup> he arrives at the following conclusion:

Sodium salicylate does not exert a favorable effect on infections in rabbits caused by various types of streptococci. It does not prevent localization of the organism in joints, nor does it prevent the appearance of endocarditis. It would seem to have, therefore, no prophylactic value, nor does it alter the course of the infection after it has once become established.

In these experiments sodium salicylate was used in a dose of 0.3 gm. administered subcutaneously once daily. In view of the great importance of the question, and the fact that the subcutaneous administration of a single daily dose by no means reproduces the usual therapeutic administration of this remedy in human beings, and as only large doses produce marked effect in human rheumatism, and the daily dose of 0.3 gm. does not represent the limit of possible dosage in the rabbit, it was thought desirable to repeat this work using a larger dose and employing various means of administration of the salicylate so as to maintain, as far as practicable, a fairly high and continuous salicylization of the system. This represents the modern ideal of administration of salicylates in the treatment of human rheumatism, based on the assumption that salicylate acts in this condition as a systemic antiseptic, not as disinfectant. It is generally agreed, as a result of clinical experience, that salicylate cannot exterminate the causative organism of rheumatic fever in the system of the patient. All that it is believed we can accomplish with salicylate is to inhibit the proliferation of the micro-organisms in the joints; and, to obtain this result, we generally need as large doses as the patient can tolerate, administered as continuously as possible for a considerable length of time. It is obvious that the experiments just cited, in which a single daily dose was administered, merely proved that the dose used was insufficient to exterminate or harm the streptococci. Such dose administered subcutaneously

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\* From the departments of Therapeutics and Experimental Medicine, University of Illinois College of Medicine.

1. Davis, D. J.: The Effect of Sodium Salicylate on Various Types of Experimental Arthritis, *THE ARCHIVES INT. MED.*, 1915, **15**, 555.

is rapidly absorbed, floods the system with a wave of salicylate, which, however, soon subsides owing to rapidity of elimination. That such treatment was ineffective proved by no means that the continuous maintenance of as high a level of salicylization of the system as possible might not have been beneficial to rabbits infected with the arthritis-producing streptococcus isolated from human patients suffering with rheumatic fever. In the work undertaken by us we attempted to induce the conditions just specified, to determine whether we could obtain any evidence of the assumed systemic antiseptic action of salicylates.

TABLE 1.—SODIUM SALICYLATE 0.5 GM. TWICE DAILY

	Gain or Loss in Weight, Gm.		Fever, Days	Lesions	Final Result
	One Week	Two Weeks			
<b>Salicylate Controls</b>	(Capsules)	(Solution)			
S 13.....	+ 45	— 20	....	.....	Survived
S 14.....	+ 5	+ 30	....	.....	Survived
S 15.....	+ 55	+ 7	....	.....	Survived
	Av. + 35	Av. + 11			
<b>Sodium Salicylate and Hemolytic Streptococcus</b>	(Solution)				
Rabbit 3.....	— 84 in 3 days		1	Gastric	Lived 3 days*
Rabbit 5.....	— 85 in 4 days		1	Gastric congestion	Lived 4 days
Rabbit 11.....	— 44 in 3 days		1	Gastric congestion	Lived 3 days
<b>Hemolytic Streptococcus Controls</b>					
Rabbit 7.....	+ 63	+ 95	5	1	Survived
Rabbit 10.....	— 34	— 98	8	2	Survived
Rabbit 12.....	— 44	—118	5	1	Survived

\* The animals treated with sodium salicylate all died, while the untreated animals recovered. That the salicylate did not kill the animals is shown by the negative result of the salicylate controls.

#### SODIUM SALICYLATE

Young rabbits weighing about 1,000 gm. were used in our experiments. The effect of the drug it was proposed to use in treatment was first studied by administering it to normal animals. Table 1 shows that when sodium salicylate was given in doses of 0.5 gm. twice daily by the stomach, it produced no markedly unfavorable results in the rabbits, excepting to retard their growth somewhat. It will be noted that administration in capsule form, used in the first week, was less unfavorable to growth than administration in solution (in 10 c.c. of water) practiced by means of the stomach tube during the second week. Nevertheless the difference was not great. Inasmuch as it is difficult to be sure that the animal gets the whole of the medicament when it is given in capsule, as rabbits chew the capsule and are likely



to reject a portion of the drug in spite of all possible care taken to prevent this, administration in solution was used in the treatment of the infected rabbits. The rabbits to be treated were injected by way of the ear vein at about the same time with equal-sized doses of the same culture of hemolytic streptococcus isolated from the tonsil of a patient suffering from rheumatic polyarthritis. It will be seen that all the treated rabbits died in from three to four days, while all the untreated animals survived though they had a course of fever and developed arthritic lesions. That the treated rabbits showed no joint lesions and had but one day of fever is not to be put to the credit of

TABLE 2.—EFFECT OF SODIUM SALICYLATE 0.3 GM. ADMINISTERED  
HYPODERMICALLY ONCE DAILY \*

Infected Controls			Infected Animals Treated with Sod. Salicylate†		
Experiment	Rabbit	Lived, Days	Experiment	Rabbit	Lived, Days
1	1	8	1	4	4
1	2	11	1	5	7
1	3	7	1	6	3
2	1	5	2	4	5
2	2	3	2	5	2
2	3	Survived	2	6	3
3	1	Survived	3	4	9
3	2	Survived	3	5	Survived
3	3	Survived	3	6	Survived
4	1	4	4	4	5
4	2	5	4	5	8
4	3	Survived	4	6	5
		5 survived 7 died			2 survived 10 died

\* Experiments of D. J. Davis.

† The influence of the salicylate was certainly not favorable.

salicylate, for they died before joint involvement and fever had time to develop. We must conclude that the administration of sodium salicylate was fatal to the infected rabbits, when used in a dose that did not prove very detrimental to the normal controls.

It may be of interest here to tabulate the final results of the experiments reported by Davis.<sup>1</sup> It will be seen by consulting Table 2, that while 60 per cent. of the infected, untreated animals died in an average of six days, 83 per cent. of the treated rabbits died in an average of 5.5 days. These figures evidently point in the same direction as those of Table 1, namely, that sodium salicylate harms rather than helps rabbits infected with streptococcus. When the dose of the salicylate

TABLE 3.—SODIUM SALICYLATE 0.3 GM. AND SODIUM BICARBONATE 0.3 GM.  
IN WATER 15 C.C. THREE TIMES A DAY

	Gain or Loss in Weight, Gm.		Fever, Days	Lesions	Final Result
	One Week	Two Weeks			
<b>Salicylate Bicarbonate Controls</b>	(Capsules)	(Solution)			
S 2.....	- 15	+ 45	....	.....	Survived
S 4.....	+ 5	-206	....	.....	Survived
S 10.....	+ 40	+ 58	....	.....	Survived
S 11.....	+ 70	+ 84	....	.....	Survived
S 0.....	-136	- 79	....	.....	Survived
	Av. - 7	Av. - 20			All survived
<b>Salicylate and Bicarbonate* and Hemolytic Streptococcus</b>	(Solution)				
Exp. 1, Rabbit 22.....	- 70	....	5	2 Pneu.	Lived 9 days
Rabbit 24.....	+ 25	- 6	11	1	Survived
Rabbit 28.....	-164	....	6	3 Pneu.	Lived 9 days
Exp. 2, Rabbit 41.....	- 60	....	6	4	Lived 7 days
Rabbit 42.....	- 98	-133	10	4	Survived
Rabbit 43.....	- 34	....	1	6	Lived 6 days
Exp. 3, Rabbit 47.....	- 55	-155	5	4	Survived
Rabbit 48.....	-132	....	0	0	Lived 4 days
Rabbit 49.....	+ 28	- 86	6	Pul. C. 4	Survived
	Av. - 62	Av. - 95	Av. 5.6	Av. 3.5	5 died 4 survived
<b>Hemolytic Streptococcus Controls</b>					
Exp. 1, Rabbit 16.....	+ 30	+240	4	2	Survived
Rabbit 17.....	+ 0	+110	8	0	Survived
Rabbit 23.....	+ 83	+261	3	1	Survived
Exp. 2, Rabbit 31.....	- 33	- 28	10	4	Survived
Rabbit 32.....	- 98	-135	7	6	Survived
Rabbit 45.....	+ 45	-137	10	4	Survived
Exp. 3, Rabbit 60.....	- 17	- 23	11	4	Survived
Rabbit 61.....	- 31	- 47	12	3	Survived
Rabbit 62.....	-104	-204	6	5	Survived
	Av. - 14	Av. - 4	Av. 7.9	Av. 3.3	All survived

\* Of the animals treated with sodium salicylate and sodium bicarbonate more than half the number died, while all the untreated, infected animals, as well as the medicated controls, survived.

was smaller or when the infections were more virulent, the unfavorable effect of the drug was less apparent.

#### SODIUM SALICYLATE AND SODIUM BICARBONATE

Inasmuch as sodium salicylate is generally administered with alkali, it seemed desirable to make a study of the effect of the combination on normal and infected rabbits. All the five normal controls (Table 3), which were given 0.3 gm. each of sodium salicylate and sodium bicarbonate three times daily, survived. The only harm the treatment did was that some of the animals lost in weight instead of gaining. All the nine untreated, infected animals survived, though all of them had a course of fever (average number of fever days about eight), almost all of them developed lesions (average number 3.3), and all of them lost in weight, excepting the animals used in Experiment 1, all of which gained in weight. This, as well as the other figures in connection with Experiment 1, shows that the virulence of the organisms used was comparatively low. In spite of this, the treated animals in Experiment 1, which were infected with the same kind of organism, had a much more unfavorable course, as will be seen by a glance at Table 3. Summarizing the three parallel experiments, we find that 55 per cent. of the treated animals died. Even though we discount the deaths with pulmonary lesions as possibly accidental, the fact still remains that the average number of lesions is greater, even though one of the treated animals (Rabbit 48) died before there was time for the development of lesions. The smaller average number of fever days (5.6) must not be interpreted as indicating an antipyretic action of the salicylates, for the four treated animals that survived had an average of eight fever days, which is the same as that of the untreated animals. Dying animals often have a low temperature for several days before death. The conclusion is inevitable that the treatment was harmful to the infected animals.

To determine the effect of sodium bicarbonate on normal and infected rabbits, to see what share it might have had in the unfavorable results produced by the combination with salicylate, an experiment was conducted, the results of which are shown in Table 4. Sodium bicarbonate is at least not harmful to the sick animals, though it certainly did not produce a noticeable improvement.

#### ACETYSALICYLIC ACID

Owing to the popularity of acetylsalicylic acid it was thought desirable to try it in an experiment; 0.33 gm. of it, which is approximately equal in salicylic acid content to 0.3 gm. of sodium salicylate, was given three times daily. The results were surprisingly bad, even in



TABLE 4.—EFFECT OF SODIUM BICARBONATE 0.6 GM. THREE TIMES DAILY

	Gain or Loss in Weight in Two Weeks, Gm.	Fever Days	Lesions
Sodium Bicarbonate Controls			
Rabbit 63.....	+135		
Rabbit 3.....	— 60		
Rabbit 59.....	—136		
	Av. — 20		
Sodium Bicarbonate* and Hemolytic Streptococcus			
Rabbit 121.....	—135	4	4
Rabbit 130.....	—205	5	0
Rabbit 131.....	—135	3	6
Rabbit 132.....	—115	6	7
	Av. —162	Av. 4.5	Av. 5.75
Hemolytic Streptococcus Controls			
Rabbit 134.....	—140	7	0
Rabbit 135.....	—297	5	14
Rabbit 136.....	—130	6	2
Rabbit 137.....	—165	3	2
	Av. —183	Av. 5.25	Av. 4.5

\* Sodium bicarbonate did not produce a noticeable improvement in the infected rabbits.

TABLE 5.—EFFECT OF ACETYSALICYLIC ACID 0.33 GM. THREE TIMES DAILY \*

	Gain or Loss in Weight, Gm.		Fever, Days	Lesions	Final Result
	One Week	Two Weeks			
Acetylsalicylic Acid Controls					
Rabbit 30.....	— 15	—160	....	Kidney pale	Lived 18 days
Rabbit 33.....	+ 65	—124	....	Kidney pale	Lived 16 days
Rabbit 34.....	—125	(—125)	....	Pneumonia	Lived 11 days
	Av. — 25	Av. —136			Aver. 15 days
Acetylsalicylic Acid and Hemolytic Strepto.					
Rabbit 146.....	—125	.....	4	15	Lived 8 days
Rabbit 147.....	—247	.....	5	17	Lived 8 days
Rabbit 148.....	—160	.....	4	15	Lived 6 days
Rabbit 149.....	—115	.....	2	17	Lived 5 days
	Av. —162		Av. 4	Av. 16	Aver. 7 days
Hemolytic Streptococcus Controls					
Rabbit 138.....	—130	(—130)	3	11	Lived 7 days
Rabbit 139.....	—220	—355	6	12	Lived 21 days
Rabbit 140.....	—125	—180	1	10	Lived 12 days
Rabbit 141.....	— 45	—168	5	3	Lived 21 days
	Av. —180	Av. —208	Av. 4	Av. 9	Aver. 15 days

\* Acetylsalicylic acid proved quite toxic to the control animals, and definitely harmed the infected rabbits.

the control animals. Table 5, which gives the results of this experiment, shows that all the control animals died. If we disregard the one that died from pneumonia, we have two animals that seemed to show kidney involvement as a result of the administration of this substance. While all the infected animals died, for we had a virulent strain to deal with in this series, the treated animals died in half the time and showed almost twice as many lesions. Acetylsalicylic acid, in equivalent dosage, appears to be much more toxic to normal rabbits than is sodium salicylate and it is harmful to infected rabbits as well.

TABLE 6.—EFFECT OF SALOPHEN 1.5 GM. DAILY IN FOOD \*

	Gain or Loss in Weight, Gm.		Fever, Days	Lesions	Final Result
	One Week	Two Weeks			
<b>Salophen Controls</b>					
Rabbit 1.....	— 41	+ 10	....	.....	Survived
Rabbit 2.....	— 47	+ 10	....	.....	Survived
Rabbit 4.....	— 0	+ 30	....	.....	Survived
Rabbit 10.....	— 5	+ 35	....	.....	Survived
	Av. — 23	Av. + 21			
<b>Salophen and Hemolytic Streptococcus</b>					
Rabbit 12.....	— 45	+ 30	1	4	Survived
Rabbit 64.....	—160	....	4	8	Lived 9 days
Rabbit 101.....	—205	....	4	12	Lived 6 days
	Av. —137		Av. 3	Av. 8	
<b>Hemolytic Streptococcus Controls</b>					
Rabbit 90.....	—130	(—130)	1	11	Lived 4 days
Rabbit 115.....	—172	—235	8	5	Survived
Rabbit 116.....	+ 3	+ 38	5	4	Survived
	Av. —101	Av. —109	Av. 5	Av. 7	

\* Salophen, while it scarcely injured the control rabbits, harmed rather than helped the infected animals.

## SALOPHEN

In all the experiments thus far reported the medicament was administered in capsule or by means of the stomach tube. In either case struggling of the animals was inevitable. To eliminate this factor, which might have an unfavorable influence on the sick animals, salophen in doses of 1.5 gm. daily, the equivalent to 0.9 gm. of sodium salicylate, was given in the food. Rabbits readily eat chopped carrots mixed with salophen, as this substance is practically tasteless. The only disadvantage of this method of administration consists in the fact that sick rabbits do not eat, and therefore do not get the medicine when it is mixed with the food. However, inasmuch as several days pass between the infection and the development of severe symptoms, the

rabbits certainly took a sufficient amount of medicine for it to have some influence on the course of the disease. That there was either no influence or else an unfavorable one is shown in Table 6.

#### COMMENT ON RESULTS

The results of the experiments reported do not permit one to escape the conclusion that in the experimentally induced arthritis of rabbits salicylates are not only worthless, but even harmful. To bring these results in harmony with the clinically established usefulness of salicylates in rheumatic fever in human beings several possibilities must be considered.

First, the *Streptococcus hemolyticus* used in these experiments might not have been the strain of streptococcus that is particularly susceptible to salicylate. It is well known that not all cases of infectious polyarthritis respond to salicylate in a specific manner. Some of these cases are quite as resistant to it as is gonococcus polyarthritis. It may therefore be desirable to repeat the experiment with a variety of different strains of streptococci. This is what Davis<sup>1</sup> did, with results that were distinctly unfavorable to the salicylate.

Second, it might be that there is a chemical difference between the system of the rabbit and that of man in its relation to salicylate, for example, a difference in the hydrogen ion concentration of the tissue fluids. If the action of salicylate in rheumatism depended, as has been suggested, on the liberation of salicylic acid in the inflamed tissues by reason of a high carbon dioxid tension in them, it might be that, owing to a greater alkalinity of the rabbit's tissue fluids, no salicylic acid is liberated in case of these animals. It would therefore be desirable to compare the reaction of the tissue fluids of rabbits with that of man. It would also be interesting to repeat these experiments on some other species of animals, such as young dogs or monkeys.

Third, the action of salicylate in human beings might be merely symptomatic, but not really curative. That there are clinicians who have this opinion is evidenced by such articles as Menzer's,<sup>2</sup> who, on the basis of personal observation of 140 patients, arrived at the conclusion that although salicylates may alleviate the symptoms to a marked degree, patients so treated are much more subject to recurrences, and in particular to deforming arthropathies, than were those handled without the use of salicylate. Miller,<sup>3</sup> on the basis of a rather extensive statistical study, concludes that "it would not appear justi-

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2. Menzer, A.: Dienstunbrauchbarkeit und Rückfalle bei Behandlung des Akuten Gelenkrheumatismus mit und ohne Antipyrese, Ztschr. f. Hyg. u. Infektionskrankh., 1911, **68**, 296.

3. Miller, Joseph L.: The Specific Action of Salicylates in Acute Articular Rheumatism, Jour. Am. Med. Assn., 1914, **63**, 1107.



fiable to refer to the salicylates as a specific in acute articular rheumatism, but rather as a drug which modifies the course of the disease, without actually shortening the period of infection." "The treated patients much more frequently relapse than the untreated." Badt<sup>4</sup> reports 2.7 per cent. of deaths among 148 cases of rheumatism treated with salicylate as compared with 0.6 per cent. of deaths in 176 cases treated without salicylate. It may therefore be that even in the human patient, as in the rabbit, salicylate harms rather than helps.

#### CONCLUSIONS

1. Sodium salicylate, when used in a dose comparatively harmless to animal controls, is decidedly detrimental and liable to be fatal to animals infected with the hemolytic streptococcus.

2. The addition of sodium bicarbonate does not lessen much the harmfulness of the salicylate to infected rabbits.

3. Sodium bicarbonate given alone is not injurious to infected rabbits, neither does it produce a noticeable improvement.

4. Acetylsalicylic acid, in equivalent dosage, appears to be much more toxic to normal rabbits than is sodium salicylate, and is harmful to infected rabbits as well.

5. Salophen has either no influence or an unfavorable one on the course of the infection.

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4. Badt: Quoted by Menzer, Footnote 2.

# ROENTGENOGRAPHY OF THE LUNGS

## ROENTGENOGRAPHIC STUDIES IN LIVING ANIMALS AFTER INTRATRACHEAL INJECTION OF IODOFORM EMULSION \*

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BALTIMORE

AND

L. G. ROWNTREE

MINNEAPOLIS

During the past thirty-two years, Rosenberg's<sup>1</sup> method of treating laryngeal and pulmonary tuberculosis by means of intratracheal injections of medicinal agents has been used to a rather limited extent only. In 1886, Sehrwald,<sup>2</sup> injecting into the trachea by "percutaneous punctures," showed that 10 to 20 c.c. of a mixture of olive oil and menthol could be thus injected without discomfort to the patient. Campbell<sup>3</sup> reported on the successful use of this method of treatment in 1895, describing cases in which he had injected as much as 100 c.c. of the oil-menthol mixture in twenty-four hours. The amount used by him at a single injection was usually 10 to 15 c.c. The results of this mode of treatment have not been sufficiently brilliant to obscure its possible dangers, and the method has not been employed extensively. The recent work of Auer and Gates<sup>4</sup> on the absorption of epinephrin (adrenalin) after intratracheal injection may give this method some of the clinical vogue which Sehrwald regarded as its legitimate due.

The studies to be reported in this paper were undertaken to acquire a clearer conception of the anatomy and physiology of the lungs in life. On the basis of the previous therapeutic use of relatively large intratracheal injections, a method was devised whereby the air passages could be filled or coated with material opaque to the Roentgen rays. As is well known, the interpretation of many shadows in Roentgen-ray plates of the lungs is limited only by the number of anatomic possibilities. It is often a matter of importance to determine whether lines in the plates represent bronchi, vessels, or fibrous tissue. Frequently, however, it is impossible to make this distinction. It is obvious that

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\* Submitted for publication Sept. 28, 1916.

\* From the Medical Clinic and Roentgen Department of the James Buchanan Brady Urological Institute, Johns Hopkins Hospital.

\* Read in part before the Society of Clinical Surgery, Baltimore, Oct. 20 and 21, 1916.

1. Rosenberg, A.: Berl. klin. Wchnschr., 1885, **22**, 449; *ibid.*, 1887, **24**, 466.

2. Sehrwald: Deutsch. Arch. f. klin. Med., 1886, **39**, 162.

3. Campbell, C.: Med.-Chir. Trans. (London), 1895, **78**, 39.

4. Auer, J., and Gates, F. L.: Jour. Exper. Med., 1916, **23**, 757.

roentgenograms of lungs before and after the intratracheal injection of material impenetrable by the Roentgen ray would give information as to the anatomic condition of the lungs, and thereby supply helpful diagnostic data.

#### MATERIAL AND TECHNIC OF INJECTION

As iodoform has been used in the local treatment of pulmonary tuberculosis, in bronchiectasis, and in putrid bronchitis, and since it was known to cast a shadow, it was the first choice. Preliminary experimentation showed that a suspension of iodoform in olive oil in thin layers definitely impeded the Roentgen rays. As a result of investigation it was found that the most satisfactory material was a 10 per cent. suspension of iodoform in olive oil. Successful roentgenograms



Fig. 1 (Plate 1-A).—Shows the entire bronchial tree completely filled with iodoform emulsion. The injection has been disseminated out into the alveoli, giving the lungs a "fuzzy" appearance.

were obtained with ordinary iodoform in olive oil, but a better suspension was prepared by grinding up 10 gm. of finely-divided, silk-screened, chemically-pure iodoform in 100 c.c. of olive oil.<sup>5</sup> No difference was observable between various samples of olive oil, but in order to obviate any irritant action from this substance, it would be best to use olive oil freed from oleic acid. Iodoform emulsion of this consistency adheres to the bronchial walls and does not too completely fill the alveoli. This property is necessary in securing sharp photographs of the injected lungs.

5. Prepared by H. A. B. Dunning of the pharmaceutical firm of Hynson, Westcott & Dunning, Baltimore.



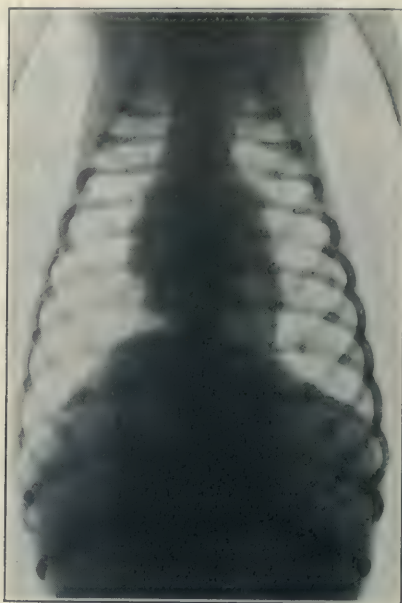


Fig. 2 (Plate 2-A).—Control; lungs before injection. Note peribronchial shadows in hilums of both lungs.

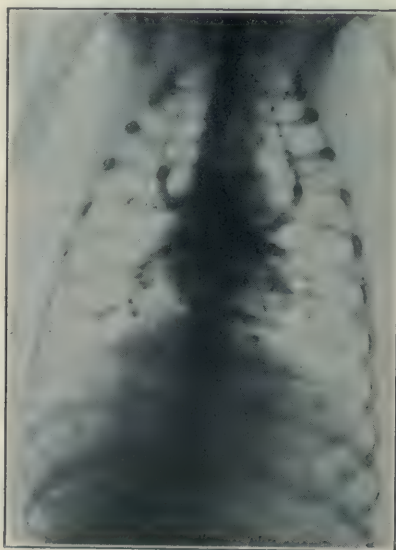


Fig. 3 (Plate 2-A).—After 10 c.c. were injected. The bronchi and bronchioles are clearly seen. This was the second injection after one month, and at the time of the injection the animal suffered from distemper, from which he died a few days later.

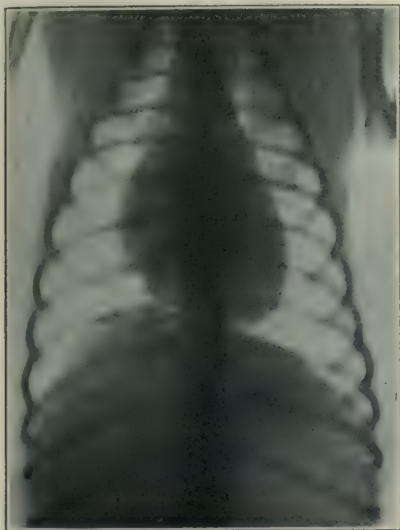


Fig. 4 (Plate 3-A, Control).—Indefinite shadows along the hilums of both lungs faintly seen.



Fig. 5 (Plate 3-A).—After 7 c.c. had been injected. Note only the bronchi and bronchioles are filled. The animal survived.

Experiments were made with the solution of thorium recently described by Burns<sup>6</sup> as a valuable agent in pyelography. The shadows cast by this fluid in the air passages were too faint and diffuse for practical value. The fluid also seemed more toxic than the iodoform emulsion.

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6. Burns, J. E.: Bull. Johns Hopkins Hosp., 1916, **27**, 157.



Fig. 6 (Plate 4-A).—Shows the right upper bronchi and alveoli well filled with iodoform emulsion. Dog choked to death.

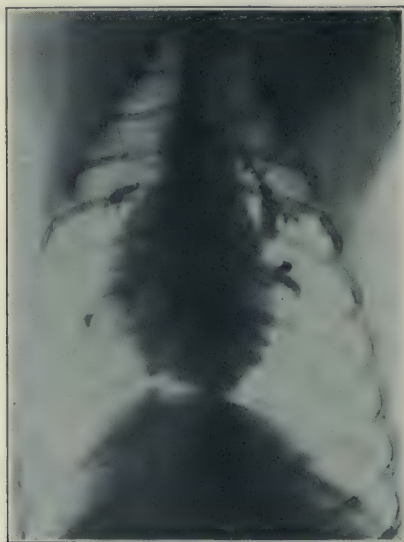


Fig. 7 (Plate 5-A).—The upper and middle bronchi well filled after 20 c.c. of the emulsion had been injected. No iodoform has passed into the alveoli.

The injections were made in the following manner:

The animal was deeply anesthetized with ether. With the animal (dog) on its back, its head retracted over the edge of the table and tongue drawn forward, a soft rubber catheter was passed through the glottis. This catheterization of the larynx is most easily made while the glottis is in full view. To secure this view a headlight was used. In order to maintain the tube in place a wooden block with a central hole for the reception of the catheter was placed between the animal's jaws. While the catheter was held in position the animal was turned on its abdomen and placed with its anterior thoracic wall



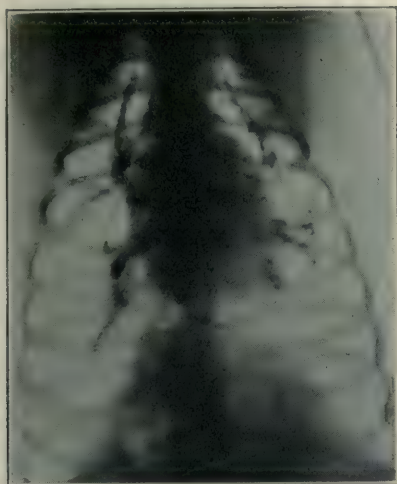


Fig. 8 (Plate 6-A).—Shows the entire bronchial tree outlined and the iodoform sticking to the wall of the upper right bronchioles.

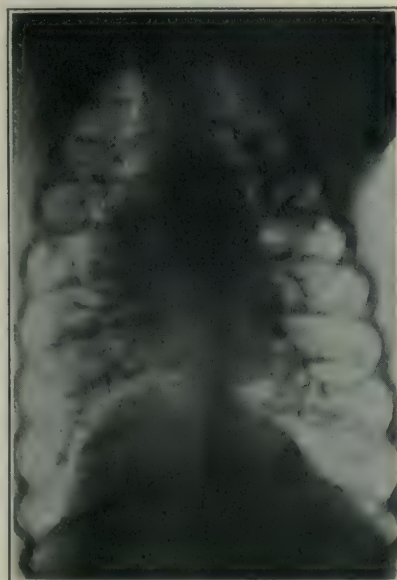


Fig. 9 (Plate 7-A).—Shows animal weighing 18 pounds after 15 c.c. of iodoform had been injected, outlining the bronchial tree and alveoli. This was the second injection; the animal survived.

resting on a Roentgen-ray plate. At the proper moment the iodoform oil was injected forcefully through the catheter by a large syringe inserted into the outer end of the tubing. At the end of the injection an instantaneous exposure was made with a soft vacuum Roentgen-ray tube placed 20 to 30 inches above the plate.

## DISTRIBUTION OF THE EMULSION IN THE LUNGS

When the lungs were dissected after an intratracheal injection of iodoform oil, this material, apparently in the same degree of suspension, was found in the bronchi, bronchioles and smallest air passages. Sections of the lungs fixed in 40 per cent. formaldehyd solution and stained with Scharlach R, showed large masses of fat filling the alveoli. The position of the catheter in the trachea determined somewhat the distribution of the fluid. As the plates show, however, the *bronchioles of the upper lobes and the alveoli of the apices were filled when the injection was made into the lower third of the trachea.*



Fig. 10 (Plate 10-A).—Shows lungs and bronchi completely filled after 25 c.c. iodoform emulsion was injected. Dog died in three minutes.

Stereoscopic roentgenograms were made on a number of these dogs, but owing to the retching following injection, it was quite difficult to keep the animal in the same position for the two exposures, and this method was soon abandoned as impracticable.

## RESULTS OF INJECTIONS

It has been possible with this method to outline the entire bronchial tree, also to demonstrate that the alveoli can be injected approximately as easily as the bronchioles. The most satisfactory roentgenograms were obtained when 15 to 25 c.c. of the emulsion were injected.

Another interesting observation was the influence of position of the intratracheal tube in determining the distribution of emulsion to alveoli and bronchioles, the best results being obtained when the end of the



Fig. 11 (Plate 17-A).—Shows the bronchial tree completely filled after 40 c.c. injection. Dog died in three minutes.

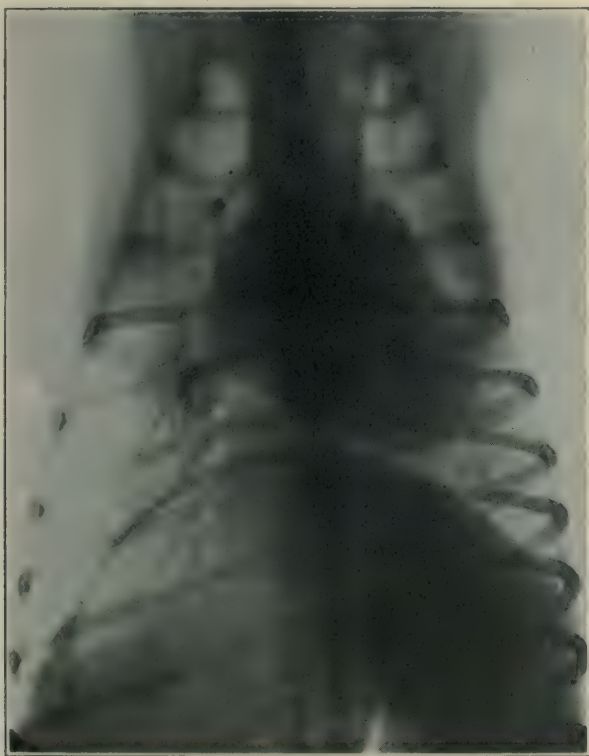


Fig. 12 (Plate 23-A).—Shows right upper and lower bronchi filled with 20 c.c. iodoform emulsion.



tube was just above the episternal notch. The bronchi leading to the apices and upper lobes were injected with as great frequency as the bronchi of the lower lobes, notwithstanding the difference in the anatomic relationship between the bronchioles of the upper and lower lobes to the course of the injection.

The accompanying table reveals the anatomic distribution of the injected fluid and shows the frequency with which each division of lung was injected.

TABLE SHOWING DISTRIBUTION—

Animals and X-Ray Plates	Position of Tracheal Tube	In Bronchi					
		Right Upper	Right Middle	Right Lower	Left Upper	Left Middle	Left Lower
1 - A.....	Medium.....	+	+	+	+	+	+
2 - A.....	Low.....	+	+	+	+	+	+
3 - A.....	High.....	+	+	+	+	+	+
4 - A.....	High.....	+	+	+	—	—	—
5 - A.....	Medium.....	+	+	—	+	+	—
6 - A.....	Low.....	+	+	+	+	+	+
7 - A.....	High.....	+	+	+	+	+	+
8 - A.....	Low.....	+	—	+	+	—	+
9 - A.....	High.....	—	—	+	—	—	+
10 - A.....	Medium.....	+	+	+	+	+	+
11 - A.....	Low.....	—	—	+	+	+	—
12 - A.....	High.....	—	—	+	+	+	+
13 - A.....	High.....	+	+	+	+	+	+
14 - A.....	High.....	+	+	+	+	—	+
15 - A.....	High.....	+	+	+	+	+	+
16 - A.....	High.....	—	—	+	—	—	—
17 - A.....	Low.....	+	—	+	+	+	+
23 - A.....	Low.....	+	—	+	—	—	—
23 - B.....	Low.....	+	—	+	—	+	+
23 - C.....	Low.....	+	—	+	+	+	+

## EFFECTS OF THE INJECTION

Twenty-two living dogs were injected intratracheally with 10 per cent. iodoform oil. Of these, three were killed in determining the toxicity or "drowning" dose of the emulsion, three were killed ten days after an injection during an epidemic of distemper in the kennels, one was killed immediately after an injection for a dissection of the lungs, two died after injections of thorium, and three died of pneumonia probably directly attributable to the iodoform injection. Ten animals

survived more than a month after the injection, and some of these survived repeated injections. The mortality among the animals therefore appears to be approximately 25 per cent., or three out of thirteen.

The type of lesion in the lungs of the dogs whose death was considered to be due ultimately to the injected emulsion, was that of a proliferative bronchopneumonia. In all cases the exudate was found to be undergoing organization, while the alveolar and bronchial epithelium was considerably hypertrophied and in several instances

## —OF INJECTED EMULSION

In Alveoli						Amount of Iodoform Oil Injected, C.c.	Weight of Dog, Kg.	Results
Right Upper	Right Middle	Right Lower	Left Upper	Left Middle	Left Lower			
+	+	+	+	+	+	25	2.7	Died in 3 minutes
—	—	—	—	—	—	10	..	Distemper when injected, died in 2 days
—	—	—	—	—	—	7	..	Survived
+	+	—	—	—	—	15	3.6	Choked to death
—	—	—	—	—	—	20	6.8	Distemper
+	+	+	—	—	—	15	7.3	Distemper
+	+	+	+	+	+	15	8.2	Survived 2 injections
—	—	—	—	—	—	8	4.5	Survived
—	—	+	—	—	—	5	4.1	Killed for pathologic study
+	+	+	+	+	+	25	..	Died in 3 minutes
—	—	+	—	—	—	15	Thorium 5.5	Pneumonia
—	—	+	+	+	+	20	Thorium 10	Died after 12 hours
+	+	+	+	+	+	15	8.2	Survived 2 injections
—	—	—	—	—	—	15	..	Survived
+	+	+	+	+	—	20	4.5	Survived
+	+	+	—	—	—	25	..	Died in 3 days—broncho-pneumonia
—	—	—	—	—	—	40	..	Drowned
—	—	—	—	—	—	20	..	Dead when injected. See Plate 23A
+	—	+	+	—	+	40	..	See Plate 23B
+	—	+	+	+	+	60	..	See Plate 23C

heaped up in two or more layers of large epithelial cells. No giant cells were seen in the sections and there was no process to suggest that the iodoform had provoked a foreign-body type of reaction.

## SUMMARY AND CONCLUSIONS

A method is described by which shadows cast by the air passages of the lungs may be unequivocally defined in roentgenograms. This method consists of injecting into the trachea a 10 per cent. suspension



Fig. 13 (Plate 23-B).—Shows same animal after 40 c.c. iodoform emulsion had been injected. The stippled shadows show that the injected material has filled up the alveoli.



Fig. 14 (Plate 23-C).—Same animal after 60 c.c. had been injected. The entire bronchial tree with the alveoli are clearly shown filled with iodoform emulsion.



of iodoform in olive oil, which renders the air passages opaque to the Roentgen ray. The limit of a safe dose of this emulsion for dogs is found to be approximately 4 c.c. per kilogram of body weight. The plates are made by an instantaneous exposure by a soft vacuum tube placed above the animal (20 to 30 inches above the plate). As similar suspensions of iodoform in oil have been used in the local therapy of human pulmonary diseases, it is hoped that perfection of the method may ultimately permit its application to man. This preliminary study is the basis of the belief of the authors that by such a method it may become possible to define with precision many radiographic shadows which are at present largely data for speculation.

## A CASE OF INFUNDIBULAR TUMOR IN A CHILD

CAUSING DIABETES INSIPIDUS WITH TOLERANCE OF ALCOHOL \*

L. NEWMARK, M.D.

SAN FRANCISCO

The clinical notes in this case are deficient in a number of particulars which those interested would wish to know, and the anatomic examination could proceed but little beyond the parts primarily involved. But defective as the following description is, it is submitted as exhibiting a good example of a rather uncommon disorder. The tolerance of alcohol in diabetes insipidus is mentioned in textbooks, but it does not appear that their statements are based on any considerable body of observations. In the reports published in recent years of cases of the disease and of experimental polyuria in animals, this subject seems not to have been considered at all.

### REPORT OF CASE

*History.*—According to information kindly supplied by Dr. Florence Scott of Belvedere, Calif., the patient, Pete B., a native of Italy, fell from a wagon when he was 4 years old and fractured his skull. He recovered completely from the effects of this accident, and it is considered certain that he did not have the unquenchable thirst before he was about 9 years old. Since the age of 6 he was addicted to smoking cigarets. To Dr. Florence Scott the child's polydipsia was revealed when she saw him at a comparatively late hour one night walking about the house with a large pitcher of water which he had left his bed to get. Inquiry elicited that the boy had been drinking very copiously and urinating large quantities every night for about a year previously. As he was at that time 10 years of age, the polyuria and polydipsia must have attracted the attention of his parents when he was about 9. His father subsequently told me that the boy drank as much as 2 gallons of water during the night, and that a bucket of a capacity of 4 gallons was placed in his bed room as a urinal and was regularly found half filled in the morning. The urine, the father added, looked like the water the child drank. Dr. Scott examined it many times and found the specific gravity generally 1.002 and never more than 1.006; sugar and albumin were always absent.

The impression obtained was that the thirst and the urinating were rather more troublesome at night than during the day; for, while there was abundant evidence that the boy drank copiously throughout the day (the customers, for instance, to whom he delivered milk, observing how frequently he drank at the faucet on his visits), yet his teacher related that he never had to be excused during lessons to drink or to urinate.

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\* Submitted for publication Sept. 22, 1916.

\* From the San Francisco Polyclinic. Thanks are due to Professor Ophüls of Stanford University, and to Professor Rusk of the University of California, for much help in pathologic matters, and to Dr. H. D'Arcy Power of the Polyclinic, for the photographs of sections.

There was no limit to the amount of water procurable to slake his thirst; but he craved stronger drink. On account of his early addiction to tobacco and a propensity to "swear dreadfully," he had for some time been viewed askance; and the measure of his iniquity seemed indeed full when the father denounced his son to Dr. Scott as a bad boy, incorrigibly given to drink. Severe beatings did not deter the boy from various tricks to obtain wine. He would drink the home-made wine from the barrel by sucking it up through a tube. To obtain beer from the grocer (who refused to sell it to him directly) he would hand his money to some vagrant and purchase through that agent. It was a matter of common knowledge in the neighborhood that P. would not recoil even from whisky. This was denied to me by the father after the child's death, but I am informed that in a recital of his complaints about the boy some years before, the drinking of whisky had been included. I have the father's word for it, however, that the boy would drink three or four glasses of beer in rapid succession, or toss off several glasses of wine. His indulgence in wine exceeded by far the measure of what some may think is permitted the young among people of Latin race. The father said he had never seen a child drink alcohol like Pete. But he had never seen the boy drunk, nor could any one else be found who had seen him affected by drink. After a bout, for instance, shared by "another little boy" who, we may safely presume, got but a small portion of the four quarts and a pint of beer consumed, he reported at school and performed his tasks well, as he usually did. His teacher rated his intellect as developed in proportion to his years, and approved his work to the last.

The headaches from which he suffered at intervals of several weeks for at least three years, were looked on as "sick headaches," and were not ascribed to indulgence in alcohol, or to grave organic disease. Generally they did not last beyond a day, and his attendance at school was not interrupted for a longer period than that, until about two weeks before his death. They were not always severe enough to make him take to his bed. He did not vomit in the attacks. The headaches over, he would resume his smoking and drinking whenever opportunity presented.

*Examination.*—In January, 1914, on a visit to the village where he lived, the subject of the foregoing history was brought to my attention as a "case of diabetes insipidus." The patient proved to be a weazen, alert and agile little boy, 14 years of age, but who looked not more than 10. He had not grown much, if at all, for a few years previously, and had always been considered small for his years. (His father is only 5 feet 5 inches in height.) On this occasion I examined the boy as well as the circumstances permitted. In view of the diabetes insipidus and its reference to lesions in or near the hypophysis, I tested especially the sense of smell, examined the backgrounds of the eyes, and sought to determine the visual fields. The result of the examination was negative.

*Second Examination and Course.*—Very different was his condition six weeks later. He was brought to San Francisco Feb. 27, 1914, because the headache, which had set in ten days before, persisted, and was attended with vomiting and a slow pulse. Now there was incipient but distinct papillitis in both eyes. Anosmia was found at the left nostril, and a few days later there was evidently a reduction of the sense of smell at the right also. The pulse rate was 53 on admission, and had been as low as 48. The headache, which was frontal, was intense and the patient was delirious a good deal of the time, and tossed about wildly in his pain; but there were intervals when the pain subsided and the mind was lucid, and during such an interval it was possible to test his visual fields again, roughly, but no hemianopsia of any kind could be made out. Ankle clonus could generally be provoked on the right, and occasionally on the left. From the sole of either foot a normal (flexor) as well as an abnormal (extensor) response could be elicited: a flexor response followed



on stroking the sole in the hollow of the foot, an extensor when the stimulus was applied to the skin over the heel. By the Oppenheim method either normal flexion of the big toe was obtained, or no response at all. In all other respects the result of the neurological examination was negative. The Roentgen ray showed a normal sella turcica. The blood counts and the Wassermann test of the serum yielded nothing worth noting. The hemoglobin was 75 by the Talqvist scale. It was decided not to do a lumbar puncture. There is no record of the blood pressure.

The body of the boy was not as lean as might have been expected from his thin visage, but there were no local accumulations of fat. According to the mother's statement, the child's appetite had been keen in the beginning of the disease and he had grown fat, but later it was ordinary. Puberty was not clearly developed. The testicles were not examined; there was nothing striking about the penis. The voice was that of a child.

On account of the vomiting, but little could be given by the mouth, but he continued to pass more liquid than the sum of what he drank and what he could have absorbed from the enemas. During the few days he was under observation in the city, the largest quantity of urine collected in twenty-four hours was 126 ounces; one voiding, however, was lost, and this may have amounted to a considerable quantity, for he urinated 24, 29, 30, 32 and 36 ounces at one time. The avidity with which he seized and drank whatever was offered to him showed the persistence of his polydipsia to the end.

The temperature in the mouth ranged, on February 27 and 28, between 98.6 and 99.7 F. At 11 p. m., March 1, the rectal temperature was 96.8. The oral temperature rose the next morning to 98.4 and fell in the afternoon to 97.8. On the morning of March 3, 96.1 was recorded by the mouth, being the lowest on that day; March 4 at 6 a. m. the mouth temperature was 95; the following morning it had risen to 96.8, and at 10 in the night of March 5 it had dropped to 94.4, the minimum of the whole course. By 5:30 in the morning of March 6 it was 95.5. About two hours later, while eating, the patient had a turn for the worse and soon expired.

*Necropsy.*—The necropsy had to be performed very hastily and incompletely, so that only the brain and the hypophysis, removed separately, could be obtained.

Nothing special was noted about the skull. The sella turcica was normal. There had been a fresh hemorrhage, no doubt the immediate cause of death. The effusion was found at the base of the brain, chiefly under the pia covering the pons and the interpeduncular space. The optic nerves were of a rather striking butter-yellow color. The anterior aspect of the hypophysis was of the same color, while the posterior was dark red. No infundibular stalk was seen.

After the brain had been hardened in formaldehyd, the corpus callosum and the fornix were removed, and the tela choroidea was reflected without any abnormal attachment being encountered. The third ventricle was then seen to be occupied by a long, dark-red tumor, into which the fatal hemorrhage had taken place. The pineal gland was very small, a thin leaflet, and might easily have been overlooked.

*Microscopical Examination.*—A series of sections was cut through the tumor in situ, and these were stained by the method of Weigert or that of Van Gieson. The Weigert sections, which are here reproduced, illustrate the extent and relations of the tumor, and may serve in place of much tedious description. They are ordered in the direction from nose to occiput.

Figure 1 shows the tumor in the space between the two hemispheres, near the base of the frontal lobes. In Figure 2 it is seen to occupy the whole space between the anterior commissure above and the optic nerves below, the latter just joining to form the chiasm. The growth is here in intimate connection with the dorsal surface of the united optic nerves. The optic chiasm appears next (Fig. 3), flattened and stretched over the basal surface of the tumor. In the planes next following, as the optic tracts diverge from the chiasm they

look in the sections as if they supported the tumor on either side of its ventral aspect; between these tracts the tumor protrudes, the tuber cinereum, normally situated here, having been destroyed. In this region, according to Professors Ophüls and Rusk, it is probable that the tumor originated, having sprung from one side of the infundibulum. In Figure 4, in a plane a little farther backward, the optic tracts are seen to have diverged sufficiently to leave the growth; the remnants of the tuber, through which the tumor has forced its way, seem to dangle at either side. The distention of the third ventricle, and the complete separation of the hemispheres at the base are conspicuous at this

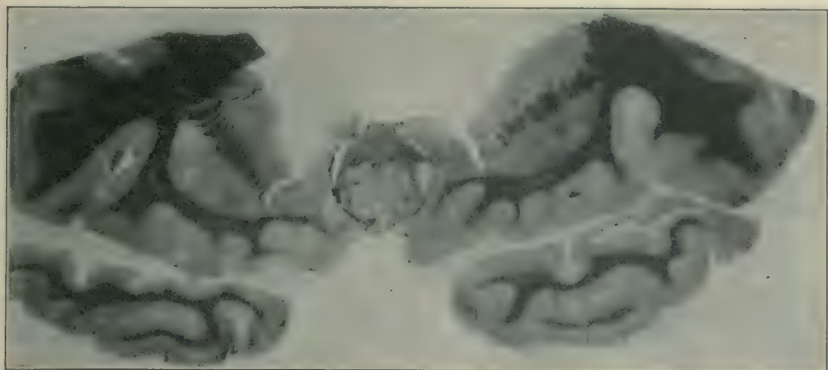


Fig. 1.—Tumor in the space between the two hemispheres, near the base of the frontal lobes.

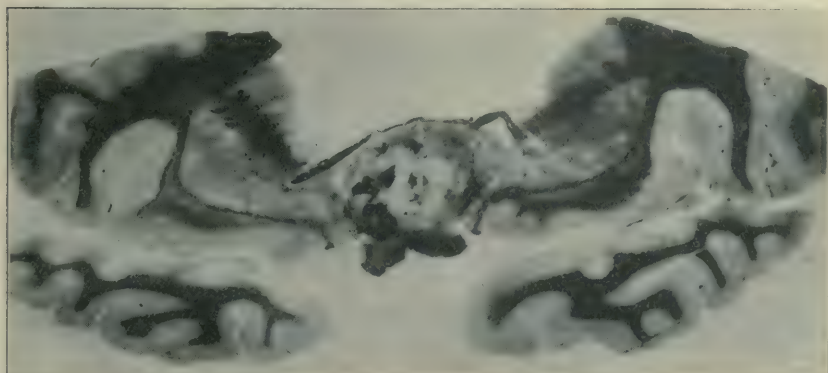


Fig. 2.—The tumor is seen to occupy the whole space between the anterior commissure above and the optic nerves below, the latter just joining to form the chiasm. The growth is in intimate connection with the dorsal surface of the united optic nerves.

level. The lenticular nuclei, the internal capsules, the descending pillars of the fornix, the structures of the optic thalami, the gray lining of the ventricle laterally to the tumor, appear pushed aside without having suffered visible damage. The section reproduced in Figure 5 passes just anteriorly to the pons, some of the pontile tissue being included on one side, and shows the tumor occupying the interpeduncular space. The pale structures situated on either side between the tumor and the peduncle are the corpora mamillaria, elongated, flattened and widely separated by the intervening growth; a thin

membrane stretched between them forms the floor of the ventricle. The same relation between the tumor, on the one hand, and the mamillary bodies, the optic thalami and the hypothalamic region on the other, appears in Figure 6, where the section passes through the pons. The growth is shown, not far from its posterior end, lying in the ventricle, in Figure 7, which illustrates a section passing through thalami, red nuclei and pons.

The tumor did not extend to the posterior commissure and the pineal body. The aqueduct of Sylvius was not dilated. No lesion was found in the medulla oblongata or fourth ventricle.

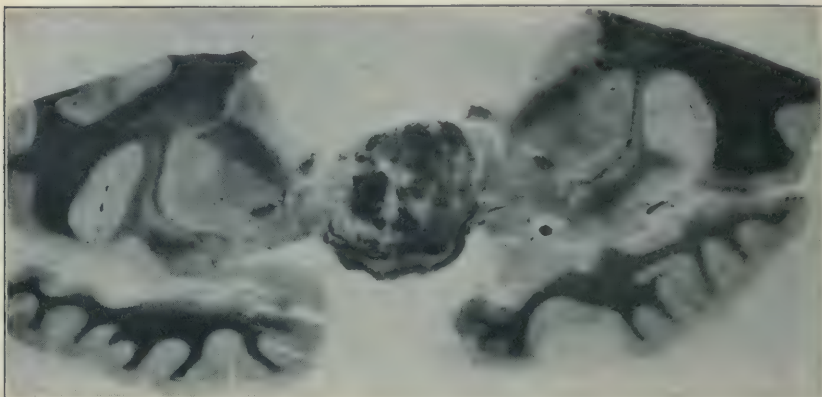


Fig. 3.—The optic chiasm appears flattened and stretched over the basal surface of the tumor.

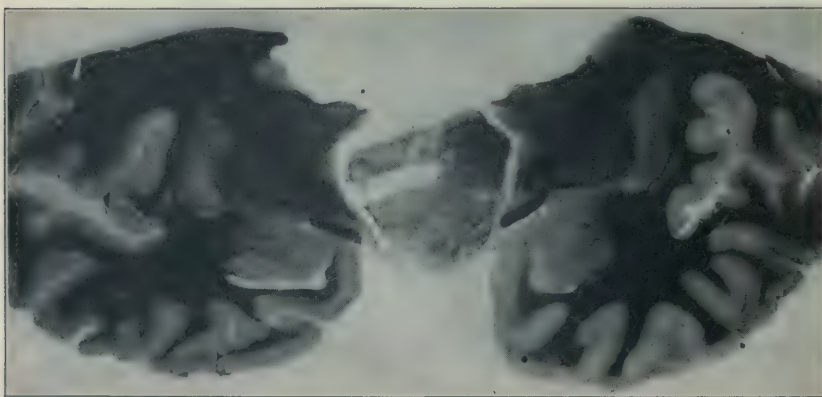


Fig. 4.—The optic tracts, which in a more frontal plane seemed to prop the tumor, have diverged from it. Note protrusion of tumor through tuber cinereum.

The dark areas in the tumor denote hemorrhage. The growth is very rich in cells, which show a distinct alveolar arrangement. There is considerable connective tissue. It has not been found possible to establish with certainty the nature of the tumor; the stronger inclination has been to consider it to be of an epithelial character. As already indicated, the infundibulum is held to be the most probable place of origin. Sections of the tela choroidea did not reveal tumor cells there.

The hypophysis: No remainder of the infundibular stalk or of the pars



nervosa could be identified. The organ consists of pars anterior and remnants of the pars intermedia. In stained sections through the region of the pars intermedia there is visible to the naked eye an area somewhat conical in shape, about 1 mm. wide and nearly 2 mm. deep, penetrating from the fibrous covering of the hypophysis into the organ; this area is conspicuous from the intensity with which it has taken the hematoxylin. The normal tissue is replaced there by a fairly cellular connective tissue, in which there are large cells with very large, very deeply staining nuclei. These cells are either polygonal or somewhat elongated. There is quite a heavy infiltration with lymphocytes throughout this tissue. Between this growth and the tissue of the anterior lobe the boundary is partly fairly well marked, but along a line it becomes ill-defined, and here the large cells of the tumor and the smaller cells of the glandular part of the hypophysis are in places intimately mixed. At the apex of the wedge of tumor tissue cysts and follicles of the pars intermedia are to be seen in fair number; they contain oxyphil colloid. The follicles are lined with clear nuclei.



Fig. 5.—See legend of Figure 4. This section passes just anteriorly to the pons, some of the pontile tissue being included on one side. The tumor occupies the interpeduncular space. See text for further description.

In anteroposterior sections beyond the region of the pars intermedia the tumor is superficial, lying directly beneath the infiltrated capsule of the hypophysis, in a shallow depression of the anterior lobe, and for the most part well defined against the hypophysis, but in one region intimately connected with it. The pars anterior contains eosinophil and chromophobe cells in the usual proportions, but is deficient in basophil cells. The fibrous capsule of the hypophysis, where it covers the tumor, contains blood resulting from a recent hemorrhage.

**Pineal Body:** This structure had the usual shape of a cone. As has been remarked, it was quite small; its length did not exceed 4 mm.; its greatest thickness was a trifle more than a millimeter and a half; its width cannot

be given in figures, but it was proportionate to the other dimensions. It had been cut off close to the habenular commissure and sagittal sections were made.

Under the microscope the most conspicuous object is an accumulation of brain sand, situated in about the middle of the organ. Between this and the apex of the conical body there is a great scarcity of cells, the meshes of the fibrous framework being for the most part empty. The fibrous tissue is hardly abnormally developed here, but there is rather more of it than in the region toward the base of the gland. Among the deposits of sand there is a group of lymphoid cells around a vessel which itself shows no changes. In the area between the concretions and the base, the cells are present in considerably larger number than toward the apex, but still are relatively sparse. The large round nuclei of the characteristic cells are chiefly of the light variety, with



Fig. 6.—Section passing through the pons. See text.



Fig. 7.—Tumor near its posterior end in third ventricle. The band of fibers on either side of the tumor is the fasciculus retroflexus of Meynert.

fine granules; sometimes the granules are arranged like a wreath around the periphery of the nucleus. There are also very pale, shadowy nuclei. Nuclei with the coarser granules are deficient. In a large transverse section through the brain stem, stained to show the medullary sheaths, a group of cells, considerable in number, is seen close to the posterior commissure; they evidently belong to the pineal body, but the method of staining precluded differentiation of them.

The pineal body, accordingly, is atrophied, and the disappearance of cellular elements is more marked in the direction of the apex than toward the base.

## DISCUSSION

Diabetes insipidus, arrest of growth and low temperature were observed in combination before there was a knowledge of their relation to the hypophysis or its neighborhood. The low temperature was ascribed to "the loss of heat resulting from the increased elimination of water and the frequent ingestion of cold fluids."<sup>1</sup> In the case before us the variations of temperature are known only as they occurred in the last days of life. There was no relation at that time between imbibition and elimination of fluids, on the one hand, and the degree of temperature on the other; rather did the regulation of temperature appear to become disturbed in accordance with the increase of congestion in, and pressure by, the tumor, and the lowest temperature, recorded on the last night might have been regarded as an omen of the final issue, which occurred the next morning.

To the units of the symptom complex mentioned should be added the tolerance of drugs, and perhaps intolerance of them. Like the lowering of the temperature, the tolerance of large doses was attributed to the activity of the urinary secretion. Thus Guinon<sup>1</sup> wrote in 1890:

One phenomenon which is associated with the activity of the urinary secretion is the immunity against alcoholic drinks and drugs. Extract of valerian in large doses, ergotin in grammes continued for several days, antipyrin in doses of a drachm and a half administered regularly, produce no bad effect on these children, because the active element of the drug is eliminated by the lavage of the tissues.

This is an obvious explanation for increased tolerance; but it would not account for the "exaggerated susceptibility" which has been asserted in regard to alcohol as well as to other substances.<sup>2</sup> Few records of original observations on diabetes insipidus in which mention is made of the effect of drugs have come to my knowledge from the older literature, and none from that of recent years; and judgment concerning the earlier cases is rendered difficult by the uncertainty of their pathology. It is improbable that all the polyurias described as diabetes insipidus have an identical pathology. For instance, what is one to assume to be the lesion in a man<sup>3</sup> 58 years of age, who was admitted with what was erroneously considered a classical alcoholic delirium, who had had polydipsia and polyuria for thirty-one years? In the history which was delivered with the patient it was stated that he tolerated alcohol badly, but the patient denied, on recovery from the

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1. Guinon, L. J.: *Urinary Neuroses of Childhood*, Wood's Medical and Surgical Monographs, 1890, p. 715.

2. Ralfe and Bradford: Chapter on Diabetes Insipidus in Allbutt and Rolleston's *Syst. Med.*, 1907, **3**, 212. Tyson's *Practice of Medicine*, Ed. 5, 1910.

3. Julius Wagner: *Vermeintliches Alkohol-Delirium, Diabetes insipidus. Salicylsäurevergiftung*, *Wien. klin. Wchnschr.*, 1888, **1**, 778.



delirium, that he had been drinking, and the diagnosis of the etiology was regarded as invalidated by the discovery of the polyuria of 7,000 to 9,000 c.c. with a specific gravity of 1.003 to 1.005 as "it was well known that patients with diabetes insipidus have a remarkably great tolerance for alcoholic drink." The delirium was thereupon ascribed to sodium salicylate, of which the patient had taken rather large doses; this had had the effect of greatly reducing the excretion of urine. The inference seemed to be that insufficient elimination had thus led to intoxication. But in a case described by Trousseau,<sup>4</sup> apparently the *observatio princeps* in this matter, there was associated with an amazing endurance of alcohol an extraordinary sensitiveness to certain other drugs. The patient in this instance was a youth of 20, whose polydipsia and polyphagia had begun at 16. "He stated that from the beginning he had acquired so great an immunity as to be able to drink large quantities of stimulants without feeling the slightest symptoms of being drunk. On several occasions he took on a wager 20 liters of wine, gaining his wager without producing any effect on the nervous system." At a time when the patient was drinking "33 liters in the 24 hours, and passing from 37 to 43 litres in the same space of time," Trousseau "tried belladonna in doses of one centigramme; and—strange to tell!—this man, who could drink 20 litres and a litre of alcohol of sp. gr. 0.835 (*90 degrés centésimaux*)<sup>5</sup> without being intoxicated, experienced violent effects from this minute dose of belladonna; and each time it was repeated similar results were produced. I then had recourse to strychnia." But strychnin was likewise not tolerated.

This was undoubtedly a severe case of primary polyuria, not a primary polydipsia of purely functional, or psychic, character. The patient was one of those who "seized the chamber-pot, and drank the contents to the last drop" while "enduring an almost total withdrawal of drinks." There had been a small quantity of sugar in the urine at times at the beginning of the malady. "Convulsive phenomena" and "failing of sight" are mentioned. All of which circumstances concur to fortify the impression of a pituitary or infundibular diabetes insipidus.

In the course of extensive, but still far from exhaustive, study of the literature I have not come on any case in which the tolerance of drugs had been observed and the cause of the polyuria had been certified by necropsy. The case with which this paper deals demonstrates that in a polyuria resulting from infundibular disease, the brain may

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4. Trousseau: Lectures on Clinical Medicine, New Sydenham Society's Translation, 1870, 3, 528.

5. The translator explains that a liter of alcohol à *90 degrés centésimaux* is rather more than a quart of alcohol at specific gravity 0.835, or 57½ overproof, which is a trifle stronger than the rectified spirit of the British Pharmacopeia.

not be affected by alcoholic draughts to which immaturity, a previous injury to the skull, and the presence of a large vascular tumor might be supposed to render it peculiarly sensitive. On what this immunity depends need not be left to speculation, but should be determined by investigation on appropriate human or experimental material.<sup>6</sup>

Abundant evidence has been collected at necropsy, as well as by experimental work, to establish the dependence of polyuria on disease localized in the region in which the tumor was found in the present case. But it has been found difficult to explain the process by which the abnormal diuresis is produced, and the very organ involved is subject to dispute. It is probably no mere coincidence that the neurohypophysis should furnish an extract with diuretic properties, and that disease in, and about, the neurohypophysis should be so often associated with diabetes insipidus. Yet the theory that the polyuria was due to an excess of diuretic substance has but little support from observations on man. Indeed, this theory yielded under the influence of postmortem data to the assumption that there was a deficiency of the normal restraint on diuresis, and this assumption seemed to be confirmed by the observation that pituitary extracts might diminish the amount of urine in normal persons as well as in patients with diabetes insipidus. One should, however, be cautious in concluding that these extracts are specific antidiuretics. The literature<sup>7</sup> of a little over thirty years ago contains accounts of remarkable effects of ergot in diabetes insipidus, and that, too, in some cases which seem from the concomitant optic atrophy or headaches to have been of intracranial origin. The therapeutic results reported there excel those which seem to be obtained by the use of pituitary extracts.

Excessive function of the pars intermedia or pars posterior, as well as diminished function of those parts, having failed to account for the polyuria, the notion of "dysfunction" has been introduced; and finally there are pathologists<sup>8</sup> and experimental investigators who reject hypophyseal pathology altogether from consideration in relation to polyuria. Thus, Camus and Roussy,<sup>9</sup> who have experimented very diligently on

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6. Ralfe and Bradford (Allbutt and Rolleston's Syst. Med., 1907, **3**, 216) mention the great fall in the amount of urine, often to normal, on the accession of pyrexia. Long ago Todd asserted that in pyrexia large doses of alcohol were borne without intoxication, lessened tolerance indicating the return of the temperature to normal. These statements conjoined might be made to support the view that tolerance of alcohol is independent of diuresis. In diabetes insipidus one would expect the tolerance of alcohol to be associated with a subnormal temperature.

7. DaCosta, J. M.: Ergot Treatment of Diabetes Insipidus, *Med. News*, Jan. 7, 1882, p. 5.

8. Erdheim: Ueber Hypophysenganggeschwülste, *Sitzungsb. d. Wien. Akad. d. Wissensch.*, 1904, **113**, 537.

9. Camus and Roussy: Diabète insipide et polyurie dite hypophysaire, *Presse méd.*, July 8, 1914, No. 54.



the production of polyuria, turn from the posterior lobe of the hypophysis as a mere "atrophied nervous fragment" and seek higher, at the base of the brain, in the gray matter of the third ventricle, the centers which regulate the assimilation of carbohydrates and the water content of the organism. According to their views, the presence of the tumor in the "opto-peduncular space" in the case I am reporting would amply account for the polyuria, for they could produce a copious and durable polyuria by a lesion in that region, even in animals which had previously been deprived of the hypophysis.

A brief survey of necropsy data will illustrate the difficulty of discerning the essential factor in the provocation of diabetes insipidus. The problem is akin to that of accounting for the relation between the hypophysis and "adipositas hypogenitalis," which Bernhard Fischer<sup>10</sup> has discussed, coming to the conclusion that this state is shown by postmortem examination in the human being to be generally the result of purely mechanical injury, by pressure, to the pars nervosa. He finds no explanation for the exceptions, that is, for the cases in which the anatomical conditions are given, but the dystrophia adiposogenitalis was absent. The pathological-anatomical conditions being about the same as those found associated with diabetes insipidus, it is striking that polyuria is mentioned in only four out of thirty-one cases of adiposity with tumors in the hypophyseal region, which were tabulated by Strada.<sup>11</sup>

(a). Any kind of pathological process, tuberculosis, syphilis, actinomycosis,<sup>12</sup> primary or metastatic tumors, or trauma, established in or near the hypophysis may provoke a polyuria. It has been supposed that the effect was the result of irritation of the pars intermedia. Von Gierke<sup>13</sup> got the impression of "chronic inflammation of the pars posterior with hyperplastic proliferation of the cells of the pars intermedia" in a somewhat obscure case with moderate polyuria. Schmorl<sup>14</sup> found in two cases of myxedema a not inconsiderable hyperplasia of the hypophysis, and especially of the pars intermedia, but does not mention polyuria.

(b). Metastatic carcinoma has in a number of instances been confined to the pars nervosa, leaving the pars intermedia intact. In a case reported by Götzl and Erdheim<sup>15</sup> there was a large tumor in the third ventricle, the relations of which to the floor of the ventricle were not

10. Fischer, B.: Hypophysis und Adipositas hypogenitalis, *Frankfurter Ztschr. f. Pathol.*, 1912, **11**, 145.

11. Strada: *Virchows Arch. f. path. Anat.*, 1911, **203**, 1.

12. Belkowski: Actinomycose de la base du crâne et des méninges. *Diabète insipide*, *Rev. de méd.*, 1911, **31**, 420.

13. Von Gierke: *Verhandl. d. deutsch. path. Gesellsch.*, 1914, **17**, 200.

14. Schmorl: *Verhandl. d. deutsch. path. Gesellsch.*, 1914, **17**, 231.

15. Götzl and Erdheim: *Ztschr. f. Heilk.*, 1905, **26**, 372.



unlike what has been described in my case, but the hypophysis had undergone only slight compression, its structure, however, being considered unchanged, apart from large foci of small cells in the posterior lobe. Diabetes insipidus had persisted for two years. Irritation of the pars intermedia, or obstruction to channels leading from the pars nervosa, was a possible effect of the tumor.

(c). But the pars intermedia, and indeed the whole hypophysis, may be destroyed by new growth, and yet the polyuria may persist to the end. It would require the extraction of a diuretic substance from such a tumor to support a chemical hypothesis, unless it could be made plausible that some other organ was stimulated to excessive vicarious production of a diuretic substance.

(d). It appears to be an obstructive or destructive process affecting the pars nervosa, not an irritative process affecting the pars intermedia, that excites the polyuria.

(e). But the polyuria and polydipsia have been known to subside after having lasted for weeks, where extensive destruction of the hypophysis by cancer had taken place,<sup>16</sup> and after having lasted for years (in Götzl and Erdheim's case) when the hypophysis was only slightly affected.

(f). There is a considerable body of observations concerning tumors and other morbid processes affecting the hypophysis which failed to produce polyuria. Simmonds,<sup>17</sup> for instance, found cancerous metastases without polyuria as well as with it, and he has also reported very advanced atrophy of the hypophysis in all its parts without the occurrence of diabetes insipidus.

Seeking for the cause of the effect in the most limited lesion, we may conclude that disease affecting the neurohypophysis may provoke polyuria (and until now it has appeared to be the most frequent provocative of it); but the effect need not be permanent, and it need not occur at all. This irregularity in the concurrence of disease of the neurohypophysis and diabetes insipidus may be a consequence of the fact that the neurohypophysis is not the only region from which polyuria and polydipsia may be elicited by disease.

While the medulla oblongata figures infrequently at present in the pathology of diabetes insipidus, growths in the pineal body have become associated with this disorder. Abnormalities of growth and sexual development having been referred to pineal tumors, a likeness

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16. Simmonds: Zur Pathologie der Hypophysis, Verhandl. d. deutsch. path. Gesellsch., 1914, **17**, 208.

17. Simmonds: Ueber sekundäre Geschwülste des Hirnanhangs und ihre Beziehungen zum Diabetes insipidus, München. med. Wchnschr., 1914, No. 4, p. 180; also, Ueber Hypophysisschwund mit tödlichem Ausgang, Deutsch. med. Wchnschr., 1914, No. 7, p. 322.

of the symptomatology of these growths to that of hypophyseal tumors has appeared, and it is enhanced by the inclusion of diabetes insipidus among the disturbances that may arise from either. Also a substance with diuretic effect has been extracted from the pineal body (of the sheep).

From the publications on polyuria in connection with pineal tumors, it will suffice to cite, first, a report by v. Hösslin<sup>18</sup> on a boy aged 9, who for several months drank and passed 20 to 24 quarts a day, the urine being of low specific gravity. Several weeks before death the diabetes insipidus ceased: an occurrence such as has been mentioned above as observed in hypophysial diabetes insipidus. A sarcoma of the pineal body was found in this case. Then there is the record of another boy<sup>19</sup> in whom the pineal tumor had been diagnosed during life: he had unquenchable thirst with enormous polyuria. In the reports of these cases which I have been able to see, the condition of the hypophysis is not mentioned; but it is mentioned in a third case, of which v. Gierke<sup>20</sup> gives an account. The patient was a victim, at 72 years, of metastatic carcinoma in the bones, kidneys, dura, and in the pineal body; six weeks before death an increase of the urine to 4 or 5 liters had occurred, and it continued until the end. At night water was drunk "by the can." Despite the cancer the corpse was adipose. There were found in the hypophysis only traces of a minute hemorrhage at the posterior border of the anterior lobe, which was not held responsible for the insipid polyuria. Apart from a slight change in the thyroid, the other glands of internal secretion were normal, so that v. Gierke thought only the pineal disease deserved consideration with respect to the polyuria.

What the relation of the pineal atrophy in my case is to the tumor in the infundibulum I am not able to determine by reference to other cases. Simmonds found no changes in the pineal body in his material of diseased hypophyses. Bartlett<sup>21</sup> describes it as enlarged in a case of adenoma of the anterior lobe of the hypophysis with destruction of the posterior.

#### SUMMARY

1. An account is given of a boy who died at 14 years after having had diabetes insipidus continuously for about five years.
2. Signs of a tumor appeared only about two weeks before death; whence the admonition against assuming the "functional" nature of a

18. Von Hösslin: München. med. Wehnschr., 1896, No. 13, p. 292.

19. Hijmanns: München. med. Wehnschr., 1913, No. 38, p. 2140.

20. Von Gierke: Hypophysis und Epiphysis bei Diabetes insipidus, Verhandl. d. deutsch. path. Gesellsch., 1914, **17**, 200.

21. Bartlett, F. K.: A Case of Acromegaly and Polyglandular Syndrome, with Special Reference to the Pineal Gland, THE ARCHIVES INT. MED., 1913, **12**, 201.

diabetes insipidus even after some years have elapsed without definite tumor symptoms.

3. A craving for alcoholic drink and unusual tolerance of it in diabetes insipidus are recorded. There is a need for more observations on the effects of drugs in this disorder.

4. A tumor occupying the region of the infundibulum, extending forward through the lamina terminalis, between the frontal lobes, and backward into the third ventricle and destroying the neurohypophysis and most of the pars intermedia, accounted for the diabetes insipidus.

5. There was also atrophy of the pineal body.

6. A survey of the literature confirms the view that a tumor causing diabetes insipidus is commonly situated in, or near, the neurohypophysis, but occasionally in the pineal body. It does not appear from clinico-pathological observations that it is overproduction of a diuretic substance that causes diabetes insipidus.



## THE REACTIVATED THYMUS\*

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"Persistent thymus" has been a term in the literature, though a term about which much skepticism has gathered. But it is gradually becoming established as a fact that the thymic parenchyma persists throughout life, and that this parenchyma may become reactivated to the extent that its secretion influences the endocrine equilibrium. The causes of such a reactivation are not known. Studies of the histologic picture afforded by such a reactivation are rare; hence the following data from a case that for want of a better term may be called thymic myasthenia.

### REPORT OF CASE

*History.*—G. N., a farmer, first seen at the age of 19, had been suffering for two years from shortness of breath. He had measles as a child; no other diseases except a headache about three to five times a year since 10 years of age. The patient vomited at the end of each attack. Formerly he was the "fattest" one in the family, but was now thin. Sleep was poor on account of a "stretching of the diaphragm"—evidently a feeling of constriction.

*Examination.*—The examination showed a short but well-proportioned young man, with no external evidence of disease. The stomach was dilated, reaching to the navel. Diastolic blood pressure was 95, systolic 130. The urine was negative, except for increased indican. The white blood cells numbered 13,600, polymorphonuclear cells 65 per cent., and large lymphocytes 33. Gastric lavage and intestinal cleansing had no effect on the subjective symptoms. Four ounces of sugar caused no glycosuria. Epinephrin (adrenalin), 1 c.c. of 1:3,200 solution, raised the temperature in twenty minutes from 98.6 to 99 F., the pulse remaining at 84. The temperature was labile and easily raised by emotional and physical excitement. The condition was not affected by the administration of thyroid extract 1 grain three times a day for one month.

Examination by surgeon (Dr. Binnie) was negative; by neurologist (Dr. Skoog), negative; roentgenogram of spine (Dr. Skinner), negative.

*Findings.*—The only important physical finding was increased submanubrial dulness, the outlines of which are shown in the accompanying photograph (Fig. 1). The roentgenogram of the chest (Fig. 2) shows an enlarged shadow in this area.

The manubrium was resected (Dr. Binnie) and some pieces of thymus removed. The microscopic study of these (Figs. 3 and 4) shows the epithelialization described by Matti and Klose as characteristic of the reactivation of the adult thymus.

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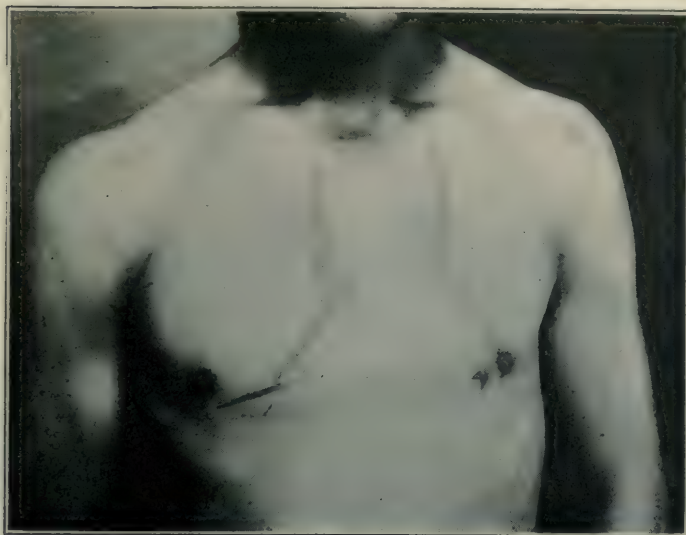


Fig. 1.—Showing the area of thymus dulness.



Fig. 2.—Roentgenogram showing the increased shadow in the thymic area.

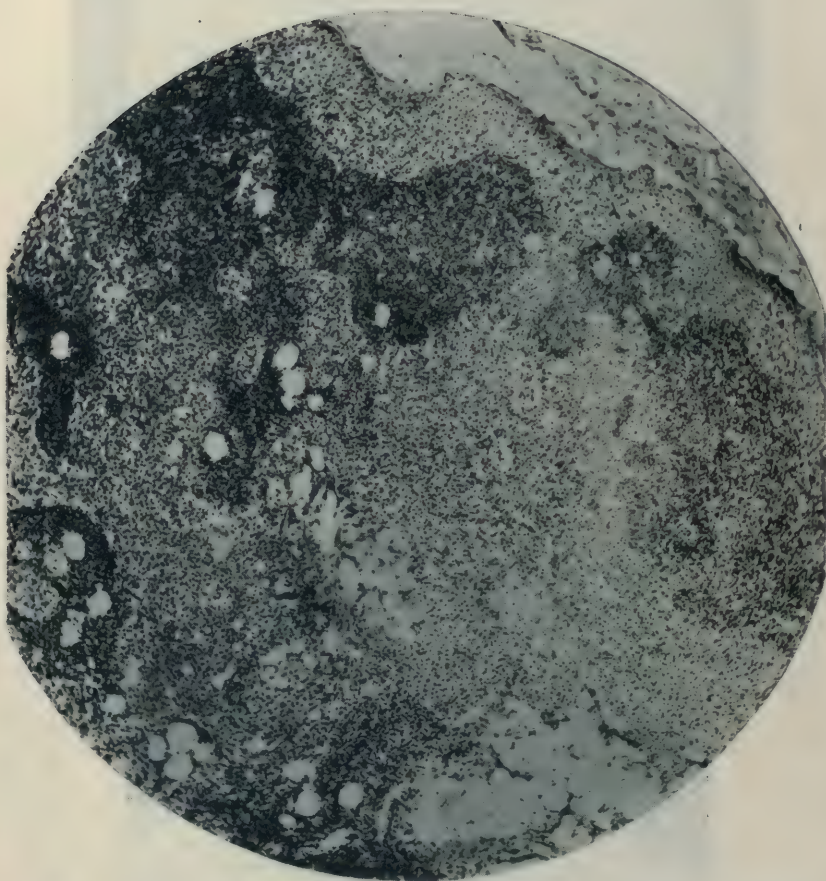


Fig. 3.—Section of thymic tissue under low power. Note the increase in the light areas, the Hassall corpuscles and the inroads of the vacuolization.



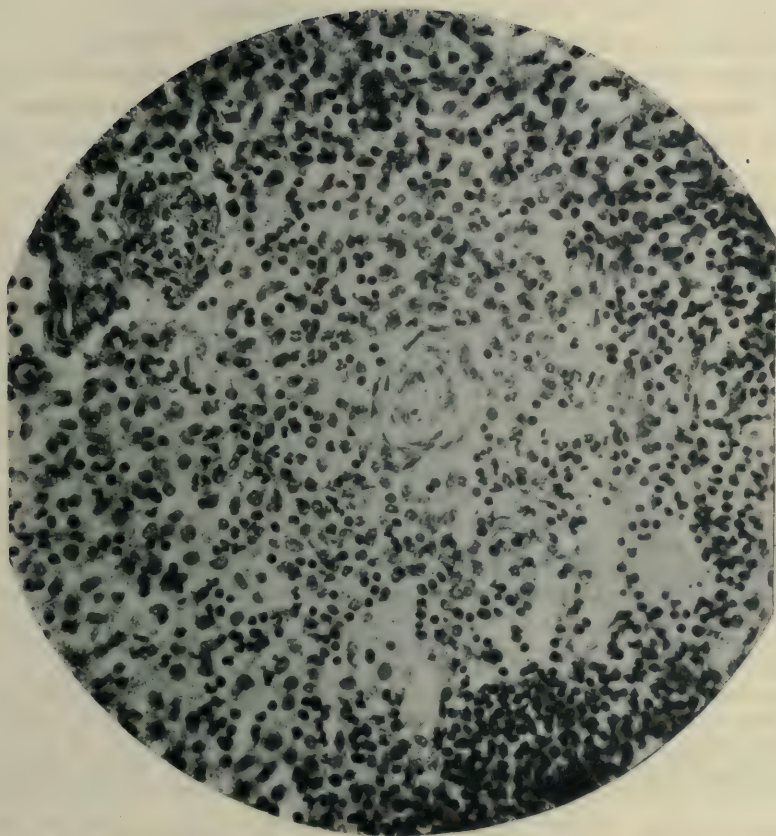


Fig. 4.—Higher magnification of the central portion of the low power field shown in Figure 3.

# A STUDY OF THE DIASTATIC ACTIVITY OF URINE AND FECES WITH SPECIAL REFERENCE TO DISEASES OF THE PANCREAS \*

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Any procedure which will enable the clinician to determine the functional condition of an organ is most desirable. From the investigations of Wohlgemuth,<sup>1</sup> Wynhausen<sup>2</sup> and Noguchi<sup>3</sup> on the quantitative estimation of diastatic activity in the blood, urine and feces, it seemed as though a method had been found which would show changes in the functional state of the pancreas. The results reported by these authors were obtained, not only in experimental pancreatic lesions in animals, but in pancreatic disease in man. Their work led to the present study.

## TECHNIC

The method employed to estimate diastatic activity was one devised by Wohlgemuth.<sup>4</sup> The principle of the method is the conversion of starch into erythrodextrin, as determined by the iodine reaction. Wohlgemuth's modification of his method has been used in our investigation because of the short time in which it can be performed.

Varying amounts of urine or a fecal suspension, decreasing by arithmetical progression from 1 c.c. to 0.1 c.c., are added to a series of ten test tubes. If a smaller quantity than 0.1 c.c. of urine is required, the urine is diluted ten or twenty times with distilled water. The test is then repeated, using the same amounts of the diluted as of the undiluted urine. To each test tube are added 2 c.c. of a 0.1 per cent. soluble starch solution. The tubes are then shaken and placed in the water bath or incubator at 38 C. for thirty minutes. At the end of that time fiftieth-normal iodine solution is added drop by drop to each tube until a color is produced. The end-reaction is considered to be present in that tube in which the blue color of starch is replaced by the reddish-brown of erythrodextrin. The units expressing the diastatic activity are the number of cubic centimeters of 0.1 per cent. starch solution, which will be converted into erythrodextrin by 1 c.c. of urine and the symbol employed is  $D_{38}^{30}$ . In working with

\* Submitted for publication Oct. 23, 1916.

\* From the Laboratories of Medicine of Harvard University, Boston, and of the State University of Iowa, Iowa City, Iowa.

1. Wohlgemuth: *Beitrag zur funktionellen Diagnostik des Pankreas*, Berl. klin. Wchnschr., 1910, **47**, 92.

2. Wynhausen: *Zur Funktionsprüfung des Pankreas*, Berl. klin. Wchnschr., 1910, **47**, 478; 1909, **46**, 1406.

3. Noguchi: *Ueber d. Fermentdiagnose bei Pankreasverletzung*, Arch. f. klin. Chir., 1912, **98**, 545.

4. Wohlgemuth: *Ueber eine neue Methode zur quantitativen Bestimmung des diastischen Ferments*, Biochem. Ztschr., 1908, **9**, 1. Wohlgemuth and Noguchi: *Experimentelle Beiträge zur Diagnostik der subcutan Pankreasverletzungen*, Berl. klin. Wchnschr., 1910, **47**, 478; 1912, **49**, 1069.

feces a filtered 10 per cent. extract in 1:1,000 sodium carbonate was used. The maximum quantity of the fecal extract was 2 c.c. instead of 1 c.c. as with urine, otherwise the technic was the same.

The original method of Wohlgemuth differs from the above-described modification in that 5 c.c. of a 1 per cent. starch solution are employed and the incubation time is twenty-four hours.

#### DIASTASE IN THE URINE OF PERSONS WITH NORMAL PANCREAS

Wynhausen,<sup>5</sup> using Wohlgemuth's original method, studied the diastatic activity in the urines of 238 patients in whom the pancreas was not diseased. He considered the normal limits to lie between 50 and 156 units in spite of the fact that the urines in six cases contained from 200 to 500 units, and in sixty-nine, less than 50 units. The majority of subsequent writers have accepted these limits, fixed arbitrarily by Wynhausen, as representing the normal.

Benczur<sup>6</sup> reported from 10 to 500 diastase units in the urines of forty-eight patients in whom the "pancreas was normal." We have collected from the literature 459 cases<sup>7</sup> without pancreatic disease, in which the diastatic activity of the urine is expressed in the units of Wohlgemuth's original method. The urines of 171, or 37 per cent., of these patients contained less than 50 diastase units. The urines of fifty-eight, or 13 per cent., of these patients contained from 100 to 200 diastase units, in eighteen cases, or 4 per cent., from 200 to 500 units, and in three cases, or 0.7 per cent., reported by Wynhausen, "more than 500 units." Cases of nephritis and of diabetes mellitus have been included in this list. The statement is frequently found that the diastatic activity of the urine is low in these two diseases. An analysis of the reported figures shows variations from "less" than 20 to "more" than 500 diastase units. Brown<sup>8</sup> using a modification of Wohlgemuth's technic, reported from 1,500 to 12,000 diastase units in the urines of forty-four normal adults. It should be noted that Brown's results are

5. Wynhausen: Quantitative Diastasebestimmungen in Harn, besonders ihre Beziehungen zur Nephritis und zum Diabetes Mellitus, *Berl. klin. Wchnschr.*, 1910, **47**, 2107.

6. Benczur: Beitrag zur klinischen Verwertbarkeit der Diastasemenge in Blutserum und Urin, *Wien. klin. Wchnschr.*, 1910, **23**, 890.

7. Wynhausen: Zur Funktionsprüfung des Pankreas, *Berl. klin. Wchnschr.*, 1910, **47**, 478; 1909, **47**, 1406; Quantitative Diastasebestimmung in Harn, besonders ihre Beziehungen zur Nephritis und zum Diabetes Mellitus, *Berl. klin. Wchnschr.*, 1910, **47**, 2107; Benczur: Beitrag zur klinischen Verwertbarkeit der Diastasemenge in Blutserum und Urin, *Wien. klin. Wchnschr.*, 1910, **23**, 890. Marino: Ueber die diagnostische Bedeutung der Diastaseausscheidung im Harn, *Deutsch. Arch. f. klin. Med.*, 1911, **103**, 325. Lindemann: Zur diagnostischen Bedeutung des Diastasegehaltes in Urin und Stuhl, *Ztschr. f. klin. Med.*, 1912, **75**, 58.

8. Brown, T. R.: The Normal Amount of Diastatic Ferment in the Urine and Feces and Its Variation in Diseases of the Pancreas, *Tr. Assn. Am. Phys.*, 1914, **29**, 547.



not comparable with the others given, as he determined the diastatic activity not for one cubic centimeter but for the entire twenty-four-hour amount of urine.

For his modified method Wohlgemuth<sup>9</sup> reports 64 units to be the maximum normal limit found for the diastase of the urine. But he does not state on what observations this conclusion was based. We have collected 216 cases<sup>10</sup> without evidences of pancreatic disease, in which the diastatic activity of the urine was estimated by Wohlgemuth's modified method. Variations from none to 66.6 diastase units were present in the urines of 208 of these patients; 100 units were present in the urines of seven patients, and 400 in one instance.<sup>11</sup>

TABLE 1.—DIASTATIC ACTIVITY IN THE URINE OF NORMAL ADULTS

Diastase Units in 1 C.c. Urine	Number of Cases
4.....	2
8.....	2
20.....	5
40.....	12
80.....	1

We have studied the diastatic activity of the urine in healthy adults and in hospital patients. Estimations of the diastatic activity were made in the urines of twenty-two healthy male adults, obtained through the courtesy of Dr. J. A. Beer of Columbus, Ohio. All but one of these urines contained less than the maximum limit of 64 units reported by Wohlgemuth.<sup>9</sup>

The diastatic activity of the urine was studied in 108 hospital patients. These patients were without evidences of pancreatic disease

9. Wohlgemuth and Noguchi: Experimentelle Beiträge zur Diagnostik subcutan Pankreasverletzungen, Berl. klin. Wchnschr., 1912, **49**, 1069.

10. Rosenthal: Zur Frage der Ausscheidung von diastischen Ferment im Urin, Deutsch. med. Wchnschr., 1911, **37**, 923. Geyelin: A Clinical Study of Amylase in the Urine, THE ARCHIVES INT. MED., 1914, **13**, 96. Rowntree, Geraghty and Marshall: A Study of the Comparative Value of Functional Tests in the Surgical Diseases of the Kidney Secondary to Obstruction in the Lower Urinary Tract, Surg., Gynec. and Obst., 1914, **18**, 201. Corbett: The Quantitative Estimation of Amylolytic Ferments in the Urine as a Measure of Certain Pathological Conditions, Quart. Jour. Med., 1912-1913, **6**, 351. Stocks, P.: The Quantitative Determination of Amylase in Blood Serum and Urine as an Aid to Diagnosis, Quart. Jour. Med., 1916, **9**, 216. Neumann: Das diastatische Ferment des Urins, Deutsch. Arch. f. klin. Med., 1913, **111**, 164. Wohlgemuth: Beitrag zum Verhalten der Diastase im Urin, Biochem. Ztschr., 1909, **21**, 432.

11. Corbett: The Quantitative Estimation of Amylolytic Ferments in the Urine as a Measure of Certain Pathological Conditions, Quart. Jour. Med., 1912-1913, **6**, 351.

or diabetes mellitus. Two cases of chronic nephritis showed 4 and 20 diastase units in the urines. In twenty-three of these cases the twenty-four-hour urines were examined; the remainder were morning specimens.

In these cases the degree of diastatic activity in the urines varied from 2 to 200 units. The same limits of variations, 2 to 200 units, were found in the twenty-four-hour urines of twenty-three patients. Seven of these (sciatica, salpingitis, secondary syphilis, hysteria, arthritis deformans) showed values of 200 units, and a case of chronic appendicitis, 100 units. Concentration of the urines played no rôle, since the quantities of urine containing the largest number of diastase units were as great as those urines containing the least number.

TABLE 2.—DIASTATIC ACTIVITY IN URINE OF PATIENTS WITH NORMAL PANCREAS

Diastase Units in 1 C.c. Urine	Number of Examinations	Number of Cases
2 to 8.....	36	33
20 to 40.....	63	63
50 to 70.....	6	5
100 to 200.....	22	7

It is evident from our own observations as well as those reported by others that the diastatic activity in the urine of patients with normal pancreas varies within wide limits; in fact, from a great diastatic activity to one so small as not to be demonstrable by this test. Five and four-tenths per cent. of our patients and 4 per cent. of those reported by Corbett<sup>11</sup> and Stocks<sup>12</sup> showed from 100 to 400 diastase units in the urine. These are figures well above Wohlgemuth's<sup>9</sup> maximum normal limit of 64 units. These variations correspond to those obtained by Wohlgemuth's original method, which showed 37 per cent. below the minimum normal limit as fixed by Wynhausen<sup>5</sup> and 4.7 per cent. well above his maximum normal limit. As in thirty-seven of our patients (24.4 per cent.) with normal pancreas the diastatic activity of the urine ranged from 2 to 8 units, and in four reported by Corbett<sup>11</sup> none was present in certain examinations, a low value cannot be regarded as evidence of pancreatic disease.

#### THE URINE IN MALIGNANT DISEASE OF THE PANCREAS

In the following eight cases the diastatic activity of the urine was higher than the usually accepted normal limits. Wohlgemuth,<sup>1</sup> using his original method, reported studies on the diastatic activity in the

12. Stocks, P.: The Quantitative Determination of Amylase in Blood Serum and Urine as an Aid to Diagnosis, *Quart. Jour. Med.*, 1916, **9**, 216.

urines of two women in whom there was "total or at least partial occlusion of the pancreatic ducts." The urine in one case contained 625 diastase units. The "different portions" of urine in the second case gave values of 625, 1,250, and then 156 to 100 units. Six hundred and twenty-five and 1,250 units in the urines of these two patients are above the maximum of 500 units found in patients without disease of the pancreas. The clinical and pathologic findings on which the diagnoses in these cases were based are not given. Whether the "different portions" of urine were collected during one or several days is not stated. No explanation is offered for the variations in the number of diastase units.

Marino,<sup>13</sup> using Wohlgemuth's original method, reported 200 diastase units in the urine of a patient with carcinoma of the pancreas, and Wynhausen, 200 and 300 units in two cases. Benczur<sup>9</sup> reported 250 diastase units in the urine of a patient with cancerous occlusion of the pancreatic duct. The diagnoses in these cases were confirmed by operation or necropsy. Using Wohlgemuth's modified method, Corbett<sup>11</sup> reported 50 to 400 diastase units in the urine from a patient with pancreatic carcinoma who came to operation. One hundred units were present in the urine of a patient reported by Stocks<sup>12</sup> in which case the diagnosis was not confirmed by either operation or necropsy.

In twelve cases of malignant disease of the pancreas the diastatic activity in the urine was "normal" or decreased. Brown<sup>8</sup> reported a case of advanced carcinoma of the pancreas in which the patient's urine contained 333 diastase units (1,500 to 12,000 units regarded as normal by his method). Using Wohlgemuth's modified method, Humphrey<sup>14</sup> found 50 diastase units in a case of "either cancer or chronic inflammation of the pancreas." Stocks<sup>12</sup> reported from 12 to 28 diastase units in the urines in four cases of malignant disease of the pancreas in which the diagnoses were confirmed by necropsy. In six cases in which a clinical diagnosis of carcinoma of the pancreas was made, but unsupported by operation or necropsy, the urines contained from 5 to 66 diastase units.

We have estimated the diastatic activity in the urines in three cases of carcinoma of the pancreas in which the diagnoses were confirmed by operation or necropsy. The stools in all the cases were very fatty. The patients were jaundiced. In two of these cases single examinations of the urine were made. The urine of one patient contained 4 diastase units and of the other, 40 units. Two samples of the remain-

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13. Marino: Ueber die diagnostische Bedeutung der Diastaseausscheidung im Harn, *Deutsch. Arch. f. klin. Med.*, 1911, **103**, 325.

14. Humphrey: The Urinary Diastase Test and Loewi's Reaction in Pancreatic Lesions, *Brit. Med. Jour.*, 1914, **1**, 1229.



ing patient were collected on different days and contained 4 and 8 diastase units.

To recapitulate: Seven cases with the unconfirmed diagnosis of malignant disease of the pancreas and ten confirmed by operation or necropsy have been collected from the literature. To the latter our three cases are to be added, making thirteen in all. Five of these thirteen patients showed an increase in the diastatic activity of the urine above the usually accepted normal limits. In one of these the diastase power of the urine was at times normal. In none of these twenty cases did the diastatic activity exceed that observed in patients without pancreatic disease. In two of our three cases and one reported by Brown, low values were obtained. But as we have shown, a low value may also be found when the pancreas is normal. Hence no importance can be attached to a low diastatic activity of the urine in the diagnosis of cancer of the pancreas.

#### THE URINE IN CHRONIC PANCREATITIS

Hirschberg,<sup>15</sup> using Wohlgemuth's original method, reported 25 to 100 diastase units in the urines in a case of chronic interstitial pancreatitis. Marino<sup>13</sup> found no diastatic power in the urine in a case of atrophy of the pancreas and 6 units in the urine in a case of slight pancreatic atrophy. The diagnoses in these three cases were verified by necropsy. Ehrmann and Kruspe<sup>16</sup> investigated a case in which the stools were large, fatty and azotorrheic. Metabolism studies showed absorption of 66 per cent. of the fat of the food. The patient was jaundiced. At operation the pancreas was found to be very hard throughout and the ductus choledochus pressed on by the head of the pancreas. Jaundice disappeared after the operation, but the character of the stools remained unchanged. Before operation the twenty-four-hour urine contained 80, 12.5, and 25 diastase units. Brown<sup>8</sup> reported three cases of "chronic pancreatitis" in which the diagnoses were confirmed by operation. The urines contained 500, 750 and 1,500 units, respectively (1,500 to 12,000 is considered the normal by his method). Corbett,<sup>11</sup> using Wohlgemuth's modified method, found 100 diastase units in the urine of a case with the clinical diagnosis of chronic pancreatitis. He reports 200 to 400 units in the urine of a case of miliary tuberculosis, showing a fibroid pancreas at necropsy, but without evidences of pancreatic disease during life. One hundred to 500 units were present in the urines of a patient with jaundice, glycosurja, diarrhea, epigastric pain, loss of weight, and stools containing 96 per cent.

15. Hirschberg: Zur Funktionsprüfung des Pankreas, Deutsch. med. Wchnschr., 1910, **36**, 1992.

16. Ehrmann and Kruspe: Untersuchungen über Pankreatitis chronica und Icterus chronicus, Ztschr. f. klin. Med., 1913, **78**, 122.

unsplit fat. This patient showed no evidences of either gallstones or carcinoma of the pancreas at operation. Spriggs and Leigh<sup>17</sup> found 20 diastase units in the urine in a case of chronic pancreatic insufficiency with fatty stools in which careful metabolic studies were made.

To summarize: eight cases with confirmed and three with the unconfirmed diagnosis of chronic, nonmalignant disease of the pancreas have been collected. The diastatic activity of the urine in eight of these cases varies from none to the usually accepted normal. Corbett's three cases were above the so-called normal limit. Corbett's case, with 500 units, is the only one in which the figure for diastatic activity is greater than we or others have found in cases without pancreatic disease. The maximum figure reported for cases without pancreatic disease is 400 units by the method used by Corbett.

The figures given for the diastatic activity in the urines in cases of cyst of the pancreas do not exceed those found by us or reported in the literature for patients without disease of the pancreas. Neumann<sup>18</sup> reported 20,000 diastase units in the urine in a case of pancreatic cyst. This figure becomes 200 units when calculated according to the terms of Wohlgemuth's modified method. Stocks,<sup>12</sup> Humphrey<sup>14</sup> and Brown<sup>8</sup> found moderate diastatic activity in the urines in five cases of cyst of the pancreas. All of these patients came to operation.

#### THE URINE IN TRAUMA TO THE PANCREAS

Noguchi<sup>3</sup> studied the diastatic activity of the blood and urine in three patients in whom injury occurred to the pancreas as a result of operative procedures. In the first case the pancreas was manipulated during the removal of a tumor from that region. Sixty hours after the operation the urine contained 64 diastase units by the modified and 160 units by the original method of Wohlgemuth. The pancreas of the second patient was superficially injured over an area about "*Fünfundzwanzigpfennig*" in size. At the end of twenty-four hours the urine showed 256 units by the modified and 320 units by the original method. A "rather large part" of the pancreas was resected in the third case. The urine twenty-four hours after the operation contained 512 diastase units by the modified and 1,280 units by the original method. The results in these three cases, together with comparable findings in experimental pancreatic lesions in dogs, to be discussed later, led Noguchi to believe that the amount of diastatic power in the urine depended on the extent of injury to the pancreas.

17. Spriggs and Leigh: A Case of Pancreatic Insufficiency, *Quart. Jour. Med.*, 1913, **9**, 11.

18. Neumann: Das diastatische Ferment des Urins, *Deutsch. Arch. f. klin. Med.*, 1913, **111**, 164.

## THE URINE IN ACUTE PANCREATITIS

Hirschberg,<sup>15</sup> using Wohlgemuth's original method, reported 400 diastase units in the urine in a case of acute pancreatic necrosis twelve hours after operation. Two hundred to 250 units were present during the succeeding two weeks and 100 to 150 units during convalescence. In a second case the urine contained 1,600 units "immediately" after operation and on the second and third days thereafter 55 and 130 units. Marino<sup>13</sup> in one case found 200 diastase units in the urine. Lindemann<sup>10</sup> made numerous estimations of the diastatic activity in the urines of three operated cases. Two were acute pancreatic necrosis and one a "retroperitoneal abscess, which reached up to the pancreas." He assumed that pressure of the abscess occluded the pancreatic duct. The diastase in the urines in these cases ranged from 500 to 1,660 units. As the patients convalesced the diastatic activity decreased, but varied between 100 and 250 units. Corbett,<sup>11</sup> using Wohlgemuth's method, reported 66 diastase units in a case of acute pancreatic necrosis in which the diagnosis was confirmed by operation. Of seven suspected cases, the urines contained 100 units in two and 50 units in five.

Using the modified method, we have estimated the diastatic power of the urines in four cases of acute pancreatic disease. The diagnoses were verified by operation. Brief reports of these cases follow:

## REPORT OF CASES

CASE 1.—*Acute pancreatitis*. This case occurred in the practice of Dr. F. J. Cotton. The symptoms were suggestive of acute cholecystitis. At the operation the peritoneum was studded with small points of fat necrosis. The lesser peritoneal cavity contained a mass of detritus and some pus. The pancreas, as such, was not distinguishable. After the operation large masses of necrotic tissue were discharged through the abdominal wound. Microscopically, this necrotic tissue was found to be largely fat. After a long period of convalescence the wound healed. Two urines in this case, collected one and two weeks after the operation, contained 40 and 100 diastase units.

The following three cases occurred on the surgical service of the Massachusetts General Hospital:

CASE 2.—*Abscess of the pancreas and acute pancreatitis*. J. A. R., aged 40, No. 193241. For two years the patient had recurrent attacks, twelve in all, characterized by abdominal distention, pain and vomiting, coming on a half to an hour after a heavy meal. The duration of these attacks varied from a few hours to a week. The last attack began five days before entrance to the hospital. Physical examination showed the abdomen to be distended. There was tenderness in the epigastrium. In the left hyochondrium was a large, rounded, smooth, slightly tender, resilient mass. The urine contained sugar. At the operation an abscess of the pancreas containing a quart of pus was opened and drained. Eight diastase units were present in the urine the sixth day after operation.

CASE 3.—*Acute hemorrhagic pancreatitis*. J. M. M., aged 40, No. 190582. The onset was sudden. There was constant severe epigastric pain and vomiting.

19. Lindemann: Zur diagnostischen Bedeutung des Diastasegehaltes in Urin und Stuhl, Ztschr. f. klin. Med., 1912, **75**, 58.



Physical examination elicited marked muscular spasm and tenderness in the right upper quadrant and epigastrium. The temperature on admission was 98.6 F.; leukocytes 27,000 per cmm. The urine was normal. At the operation the head of the pancreas was found to be hard, swollen, edematous and red. Throughout the illness the temperature varied from 98.6 to 100 F. The drainage wound closed after two weeks. The urine one week after the operation contained 20 diastase units.

CASE 4.—*Acute hemorrhagic pancreatitis*. J. F. Mc., aged 53, No. 190582. The onset was sudden. At first there were moderately severe colicky pains in the epigastrium. Within twenty-four hours the pains became very severe and were accompanied by vomiting. Physical examination showed tenderness and muscular spasm in the upper half of the abdomen. This was most marked in the epigastrium. The admission temperature was 100 F.; leukocytes 27,000 per cmm. At the operation, August 7, the pancreas was found to be a bloody, edematous mass. The patient lived one month. During this time the drainage wound constantly discharged. The temperature during the illness varied from 99 to 103 F. The urine examinations are given in Table 3.

TABLE 3.—DIASTATIC ACTIVITY OF THE URINE IN HEMORRHAGIC PANCREATITIS \*

Date	Amount Urine, C.c.	Diastase Units in 1 C.c. Urine	Fehling's Solution	Glucose in Urine, Gm.	Diastase Units in 1 Gm. Feces
8/9	Morning specimen	40	Reduced		
8/9	12-hour specimen	26	Reduced		
8/11	1,360	6	Reduced	37.8	
8/12	500	8	Reduced	10.4	
8/16	Single specimen	200	Not reduced	....	10
8/18	Single specimen	20	Not reduced	/	

\* Operation August 7.

Eight cases of suspected acute pancreatitis and six with the diagnosis confirmed by operation have been collected. To the latter we add four cases. Two cases of direct operative injury to the pancreas are reported by Noguchi.<sup>3</sup> Of the ten cases of acute pancreatitis with confirmed diagnoses, the diastatic activity in the urines in seven was on certain examinations higher than the usually accepted normal. In the urines of two patients the figures for diastase units were higher than those reported either in our own series or in the literature for patients without evidences of pancreatic disease. In the latter case 500 units are the maximum reported, while in Lindemann's<sup>10</sup> two cases of acute pancreatitis, 1,660 units were present. One of our patients showed values of 100 units two weeks after operation and another 200 units ten days after. These figures are not higher than found in 7 per cent. of our series without pancreatic disease, and in 4 per cent. collected from the literature. Lindemann assumes that the high diastatic activity in the urine of his patient with "retroperitoneal abscess" was due to the pressure of the abscess on the duct of the pancreas.

On the other hand, Noguchi's<sup>3</sup> case, in which the pancreas was directly manipulated but not noticeably injured, showed no increase in the diastatic power of the urine. In his two cases with direct injury to the pancreas the figures given for the diastase units by the modified method are high. Unusually high figures were obtained in but one of these cases. Thus of the thirteen cases of acute pancreatic affection five give figures for the diastase which are higher than found in cases without pancreatic disease. In these cases the figures given are 512 units by Wohlgemuth's modified method in one case, and from 1,600 to 1,660 units by the original method in four cases.

#### SUMMARY OF THE CLINICAL STUDIES ON THE URINE

The diastatic activity of the urine has been estimated by Wohlgemuth's modified method in 346 patients without disease of the pancreas; 95.6 per cent. of these cases showed from zero to 80 diastase units in the urines. In 450 cases without pancreatic disease, the diastatic activity of the urine was estimated by the original method; 95.3 per cent. of these cases showed from zero to 200 units in the urine.

The diastatic activity of the urine was determined by one of Wohlgemuth's methods in sixty-one cases in which the pancreas had been diseased, injured, or manipulated. This number includes our cases and those collected from the literature.

In forty-two of these the diagnoses were confirmed by operation or necropsy. In five the figures given for diastatic activity in the urine are greater than those reported for patients without pancreatic disease. In Noguchi's<sup>3</sup> case of resection of part of the pancreas the patient showed 512 units, while 400 units<sup>1</sup> is the highest reported for that method in cases without pancreatic disease. Lindemann's<sup>19</sup> case of "retroperitoneal abscess reaching up to the pancreas" showed 1,660 diastase units in the urine. Hirschberg's<sup>18</sup> one case and Lindeman's<sup>19</sup> two cases of acute pancreatitis gave 1,600 and 1,660 diastase units in the urine, while the maximum figure obtained in cases with normal pancreas by the method used by these investigators is 500 units.<sup>20</sup>

The diagnosis of pancreatic disease was unconfirmed by operation or necropsy in nineteen cases. The figures given for the diastatic activity of the urines do not exceed those found in cases without pancreatic disease, except in two cases reported by Wohlgemuth<sup>21</sup> with "total, or at least partial, occlusion of the pancreatic duct," in which the urine contained 1,250 and 625 units.

In seventeen cases, or 40.5 per cent. of the forty-two cases with

20. Wynhausen: Zur Funktionsprüfung des Pankreas, Berl. klin. Wchnschr., 1910, **47**, 478; 1909, **46**, 1406. Geyelin: A Clinical Study of Amylase in the Urine, THE ARCHIVES INT. MED., 1914, **13**, 96.

21. Wohlgemuth: Ueber eine neue Methode zur quantitativen Bestimmung des diastatischen Ferments, Biochem. Ztschr., 1908, **9**, 1.



verified diagnoses, a high diastatic activity was present in the urines. From 100 to 512 diastase units were present in the urines of seven of the cases studied by Wohlgemuth's modified method. By his original method the urines in seven cases contained from 250 to 1,660 diastase units and in three cases contained 200 units. As a high diastatic activity occurred in the urines in from 4 to 7 per cent. of cases without evidences of pancreatic disease, this finding is of limited value as an aid in the diagnosis of disease of the pancreas.

#### THE URINE IN EXPERIMENTAL PANCREATIC LESIONS

Wohlgemuth<sup>22</sup> describes the effect on the diastase in the blood and urine of tying off the principal pancreatic ducts in three animals. An increase in the diastatic power of the urine occurred within twenty-four hours and persisted several days. In the first animal, a rabbit, 40 diastase units were present in the urine before the operation of tying the ducts. During the six days following the operation 500, 780, 500, 780, 200 and 40 units were found. In the second animal, a dog, the urine possessed no diastatic activity before the operation. During the succeeding six days 80.5, 625, 312.5, 805, 10 and 5 units were present. In another dog a "small portion" of the tail of the pancreas was tied off. Before the operation the urine contained 10 diastase units and during the five days following the operation 50, 200, 125, 50 and 20 units were present.

In two dogs the ductus choledochus was tied and cut. Before the operation the urines contained no diastatic power. The figures for the diastase units the nine days following the operation are 0, 0, 0, 5, 80.5, 625, 156, 40 and 20 units for one dog, and 5, 5, 20, 10, 40, 156, and 20 units for the other dog.

These results at first sight seem to indicate that some factor is involved in producing an increase in the diastatic power of the urine other than that of injury to the pancreas. Wohlgemuth offers two possible explanations. One is that the pancreatic duct may have been closed through the formation of scar tissue. But it would not be possible for newly formed connective tissue to constrict the pancreatic ducts in such a short time. Further, he suggests that there may be some nervous relation between the ductus choledochus and the pancreatic ducts which results in a functional constriction of the pancreatic ducts when the bile duct is tied. This hypothesis is unsupported by any evidence. As an increase in the diastatic activity of the urine is known to result from acute pancreatitis, it is more probable that some inflammation of the pancreas secondary to infection about the common duct explains the rise noted.

22. Wohlgemuth: Beitrag zum Verhalten der Diastase im Urin, *Biochem. Ztschr.*, 1909, **21**, 432.



Wohlgemuth and Noguchi, using the modified method, studied the increase in diastatic power in the urine in relation to the extent of trauma produced on the pancreas of two dogs. In the first animal the pancreas was cut across. The urine contained no diastatic power before the operation. Five hourly examinations following the operation showed 8, 16, 16, 32, and 64 units. In twenty-four hours 256 units, and in forty-eight hours 512 units, were present.

The second animal's pancreas was traumatized by making a small stab wound with a scalpel and lightly squeezing the neighboring pancreatic tissue with the fingers. Before the operation 2 diastase units were present in the urine. Six one-hour examinations after the operation showed 2, 2, 8, 16 and 16 units. Thirty-two units were present in a specimen collected at the end of twenty-four hours, and 32 at the end of forty-eight hours. The authors conclude that the time of appearance and magnitude of the increase in diastatic power of the urine varies directly with the extent of trauma in experimentally-produced lesions of the pancreas. But it is to be noted that Wohlgemuth<sup>22</sup> found variations in the diastatic activity in the urines of normal dogs of from none to 78 units, using his original method.

We have studied the diastatic activity in the urines of nine dogs. In six of the animals the pancreas was traumatized. In one a pancreatic graft was placed in the spleen. Two dogs were narcotized and no operative procedures carried out. The results obtained were as follows:

Dog 1.—Female; weight 12.5 kilos; ether anesthesia; operation August 16. All of the pancreas except the processus uncinatus was removed. The dog rapidly declined in strength and dropped to 9.3 kilos in weight the fourth day. A severe glycosuria developed on the third day (Table 4). No diastatic activity was present in the duodenal contents obtained after death, August 22.

TABLE 4.—DOG 1. OPERATION AUGUST 16

Date	Amount of Urine in 24 Hours	Diastase Units in 1 C.c. Urine	Diastase Units in 24-Hour Urine	Diastase Units in 1-Gm. Stools
8/13	20	0		
8/14	40	0	.....	7
8/16	1,000	2	2,000	10
8/17	100	0.66	66	
8/18	250	40	10,600	
8/19	400	100	40,000	
8/20	330	8	1,200	
8/21	150	8	1,200	
8/22	600	0	.....	10

Dog 2.—Female, weight 4 kilos; ether anesthesia; operation August 15. Double ligatures were placed around the pancreas at the junction of the processus lienalis and corpus, and of the processes uncinatus and corpus.

TABLE 5.—Dog 2. OPERATION AUGUST 15

Date	Amount of Urine in 24 Hours	Diastase Units in 1 C.c. Urine	Diastase Units in 24-Hour Urine	Diastase Units in 1-Gm. Stools
8/11	450	2	900	10
8/14	....	1	.....	40
8/16	20	20	400	
8/17	150	200	30,000	
8/18	250	200	50,000	
8/19	200	40	8,000	10
8/21	300	0		
8/22	300	4	1,200	
8/23	300	4	1,200	

Dog 2 was subjected to a second operation under ether anesthesia, August 27. The pancreas was found to be firmer to touch than normally, but was macroscopically otherwise unaltered. A small piece of pancreas 3 cm. in length was excised and another portion was placed in a pocket made in the spleen.

TABLE 6.—Dog 2. OPERATION AUGUST 27

Date	Amount of Urine in 24 Hours	Diastase Units in 24-Hour Urine	Diastase Units in 1 C.c. Urine
8/28	15	800	50
8/29	82	164	2
8/30	70	46	0.66
8/31	400	800	2

Dog 3.—Female; weight 8.5 kilos; operated on under ether anesthesia, August 27. Three pieces of pancreas, freshly excised from another dog, were imbedded in the spleen. The pancreas of Dog 3 was not manipulated.

TABLE 7.—Dog 3. OPERATION AUGUST 27

Date	Amount of Urine in 24 Hours	Diastase Units in 24-Hour Urine	Diastase Units in 1 C.c. Urine
8/28	50	.....	0
8/29	10	1,000	100
8/30	550	2,200	4
8/31	550	2,200	4

That pieces of pancreatic tissue imbedded in the spleen rapidly undergo autolysis Pratt and Murphy<sup>23</sup> have found. The experiment on Dog 3 clearly shows that destruction of foreign pancreatic tissue may produce an increase in the diastatic activity of the urine.

In Dog 5 all of the pancreas but a portion 3.5 by 3 cm. of the processus uncinatus was excised. A pancreatic graft 3 by 5 cm. was placed in the spleen and an emulsion of pancreatic tissue also injected into the spleen. No rise in diastatic activity in the urine occurred in the five days succeeding the operation.

Two dogs from which all of the pancreas but the processus uncinatus was removed showed no diastatic power in the urines for the forty-eight hours succeeding the operations. From the second to the fifth day following a similar operation in another dog the diastase values were 10, 4, 2, and 1 units per c.c. of urine.

The effects of ether anesthesia on the diastatic activity of the urine were studied. Two dogs were narcotized with ether for two and a half hours. The urines for the following twenty-four hours contained 30' and 20 diastase units per c.c., and those on the succeeding day 1 and 4 units. As Wohlgemuth,<sup>22</sup> using his original method, found 7.8 to 78 diastase units in the urine of a normal dog, it is possible that the figures found in our two narcotized dogs represent nothing more than normal variations, but it is more probable that the administration of ether was the cause of the relatively high diastatic values.

In two of our animals (Tables 4 and 5) an increase in the diastatic activity of the urine occurred in from one to two days following injury to the pancreas. This increase persisted from one to four days.

Our findings are materially different from those of Wohlgemuth and Noguchi. They observed an increase in the diastatic activity in the urine in every animal examined in which the pancreas was injured, while none was present in the urine of two of our dogs. In all of their experiments the maximum increase occurred on the second day after the operation, while in our series Dog 1 had the greatest increase on the third day and Dog 2 on the first day after the second operation. The increase was less in our experiments than in those reported by these investigators. Only one of our dogs had 200 units of diastase in the urine. Wohlgemuth observed a rise to 805 in one of his experiments and 780 in another.

#### DIASTATIC ACTIVITY OF THE FECES

The diastase found in the feces is probably a product of the secretion of the pancreas. The exclusion of the pancreatic juice from the intestines is followed by a marked diminution of the diastatic activity

23. Pratt and Murphy: Pancreatic Transplantations in the Spleen, Jour. Exper. Med., 1913, **17**, 252.



of the feces. This has been demonstrated by the experimental work of Wohlgemuth,<sup>1</sup> Wynhausen,<sup>2</sup> and ourselves. That ptyalin of saliva is not a source of the diastase in the feces has been shown by Wohlgemuth.<sup>1</sup> This investigator tied the pancreatic ducts in dogs. After the operation no diastatic activity was demonstrable in the feces. He then fed the dogs human saliva, but the stools still remained free from diastatic activity. Fuhrmann<sup>24</sup> and Lifschütz<sup>25</sup> have shown that the bacteria of the feces can produce but a very little diastase.

It has been considered probable that the estimation of the diastatic activity of the feces would give information regarding the functional condition of the pancreas. Destructive lesions of the pancreas or occlusion of the duct of Wirsung would be expected to be accompanied by a decrease in the amount of pancreatic juice entering the intestines. This has led investigators to attempt to establish a minimum normal for the amount of diastatic activity possessed by the feces.

Wynhausen<sup>2</sup> reported a minimum of 500 diastase units and a maximum of 20,000 units from the study of 170 stools. He considers 500 units the minimum normal limit. Hirschberg<sup>15</sup> states that "a number of patients in whom the pancreas was normal" gave from 200 to 500 units of diastase in the stools. Lindemann<sup>10</sup> found a minimum of 100 units and a maximum of 2,282 units in the stools of twenty-four patients without evidences of pancreatic disease. Bálint and Molnár<sup>26</sup> state that "investigations on normal persons show great variations" in the diastatic activity of the stools, but give no figures. They report three cases of Basedow's disease in which the diastatic activity of the stools ranged from 300 units to 100,000 units. Hirayama<sup>27</sup> reported from zero to 150 diastase units in the stools in twenty-nine patients without pancreatic disease. Friedmann<sup>28</sup> studied the diastatic activity in the feces of seventeen patients without disease of the pancreas. The figures given are 25 to 50 diastase units in four cases, 250 to 600 units in four, 5,000 units in seven, and 50,000 units in one. Arnold<sup>29</sup> found from 125 to 780 units in the feces in six cases of intestinal fermentative dyspepsia without evidences of diseases of the pancreas. All of

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24. Fuhrmann: Vorlesungen über Bakterienenzyme, Jena, 1907, 84.

25. Lifschütz: Zur Frage der funktionellen Diagnostik der Pankreaserkrankungen, Arch. f. Verdauungskr., 1913, **19**, 562.

26. Bálint and Molnár: Zur Pathogenese der Diarrhöen bei Morbus Basedowii nebst Bemerkungen über diagnostische Bewertung des Fermentsgehalts der Faeces, Berl. klin. Wchnschr., 1910, **47**, 1620.

27. Hirayama: Ueber den Gehalt der Faeces an tryptischen und diastatischen Ferment beim normaler Verdauung im Fieber und im diarrhöischen Stuhl, Ztschr. f. exper. Path. u. Therap., 1911, **8**, 624.

28. Friedmann: Quantitative Estimation of Trypsin and Amylase in the Feces and Duodenal Contents, Med. Rec., New York, 1912, **81**, 355.

29. Arnold: Ueber den Diastasegehalt der Fäces bei Gärungsdyspepsie, Zentralbl. f. inn. Med., 1913, **1**, 34, 1.

these investigators used Wohlgemuth's original method. Rotky,<sup>30</sup> writing in 1913, considers the establishment of a normal as impossible from the results given by different authors.

Altogether, figures for the diastatic activity of the stools of 350 patients have been collected from the papers of Wynhausen,<sup>2</sup> Hirschberg,<sup>15</sup> Lindemann,<sup>19</sup> Bálint and Molnár,<sup>26</sup> Hirayama,<sup>27</sup> Friedmann,<sup>28</sup> and Rotky.<sup>30</sup> No diastatic activity was present in the stools of nineteen of these patients.<sup>27</sup> In the stools of seven patients there were from 25 to 50 diastase units. In the stools in 302 cases from 200 to 20,000 diastase units were present. Friedmann<sup>28</sup> reported 50,000 diastase units in the stools in one case, and Bálint and Molnár<sup>26</sup> 100,000 in another. The stools in forty-one, or 11.7 per cent. of the 350 cases, contained from none to 200 diastase units. Brown<sup>8</sup> modified Wohlgemuth's technic and studied the diastatic activity in the feces of fifteen healthy men and women. By Brown's method the number of diastase units contained in the entire amount of feces collected is computed. Variations of from 60,000 to 240,000 diastase units were found. He considers 60,000 units as representing the minimum normal limit. Brown's method consists of a special meal of 750 c.c. of milk given at 7 a. m., and followed by  $\frac{1}{2}$  ounce of magnesium sulphate at 7:30 a. m., and again at 8 a. m. At 8:30 a. m. a glass of water containing  $\frac{1}{4}$  teaspoonful of sodium bicarbonate is taken. The stools are collected up to 2 p. m., and if less than 400 gm. are obtained, an enema of a pint of water is given. The preceding evening the patient receives a high enema and is allowed only a very light supper.

The feces are collected over a period of seven hours. Certainly stools passed within the first few hours cannot result from the milk ingested. We found large amounts of vegetable fiber in the stools of two patients on whom Brown's method had been carried out. This came from vegetables eaten the day previous to the test. It is questionable how often the milk reaches the rectum in the seven hour period. Roentgenographers consider that it takes the bismuth meal used in gastro-intestinal studies from seven to nine hours to pass into the cecum. Hertz<sup>31</sup> has shown that the magnesium sulphate does not affect the motility of the small intestines, but acts only on the colon. Hence, by Brown's technic, the magnesium sulphate will have spent its effect on the colon before the milk meal has reached the cecum. To test this point the technic of the method was carried out on three normal adults. Forty grams of bismuth were added to the milk taken by two of these. Roentgenograms at the end of seven hours showed the bismuth to have

30. Rotky: Ueber den Diastasegehalt der Faeces, München. med. Wchnschr., 1913, **60**, 2158.

31. Hertz, A. F.: Constipation and Allied Intestinal Disorders, London, 1909, pp. 9 and 279.



reached the ileocecal valve in one, and to have just entered the transverse colon in the other case. Enemata at the end of eight hours in both these cases produced stools without bismuth. Seventy-five grams of bismuth were added to the milk given to the third normal person. At the end of seven hours a roentgenogram showed that the bismuth had reached the sigmoid colon, but the major part of the bismuth was in the terminal ileum and cecum. An enema at the end of seven and a half hours yielded a stool which contained no bismuth. Brown's method was also carried out without the use of bismuth on three adults who had no gastro-intestinal disturbance. One gram of charcoal was added to the milk. At the end of seven hours the stools of only one patient contained charcoal. But if a stool resulting from the milk is obtained it will be mixed with (1) the contents of the colon not removed by the high enema of the preceding evening; (2) the unabsorbed residue of the light supper; (3) and often the remains of food in the stomach or small intestines eaten prior to this meal.

Using Wohlgemuth's modified method, Stocks<sup>12</sup> estimated the diastatic activity in the stools of a normal person and in two cases of diabetes mellitus. A stool from the normal man contained 38 diastase units. Three stools were collected on different days from one of the diabetics. The stools of this patient contained 21.3, 750, and 434 units. The stools of the other contained 5 units. Neumann<sup>18</sup> found no diastatic activity in the stools in a case of catarrhal jaundice.

We have estimated the diastatic activity of the stools of sixty-eight patients without evidences of disease of the pancreas. These cases embrace fifty-two nonfebrile and two febrile diseases, that is, typhoid fever and pulmonary tuberculosis. The diet of fifty-seven of these patients was the "general hospital diet." The stools were the result of either magnesium sulphate or castor oil. In four cases the stools were diarrheal. The results are given in Table 8.

TABLE 8.—THE STOOLS OF PERSONS WITH NORMAL PANCREAS

Diastase Units in 1 Gm. Feces	Number of Examinations	Number of Cases
0.....	10	7
7 to 10.....	18	12
20 to 50.....	20	13
200 to 500.....	17	17
1,000 to 5,000.....	13	13

The stools in seven cases, or 13.2 per cent. (sprue, hepatic cyst, intestinal infantilism, chronic nephritis, typhoid fever), contained no diastatic power. In thirty-two of these cases, or 51.6 per cent., the



stools contained less than 50 diastase units. Stools collected on different days were examined for the diastatic activity in thirteen cases. It was found that variations exist in the diastatic power of different stools from the same case. That this variation is often great is shown in Table 9.

#### THE FECES IN MALIGNANT DISEASE OF THE PANCREAS

Using Wohlgemuth's original method Wynhausen<sup>2</sup> reported 10 to 30 diastase units in the stools in two cases of carcinoma of the pancreas. The diagnoses in both cases were confirmed by necropsy. Wynhausen<sup>2</sup> found 12.5, 20 and 30 diastase units in the "different" specimens of feces from a patient with a tumor in the head of the pancreas. At operation this tumor was found to occlude the duct of Wirsung. The character of the tumor is not stated. Using his own technic, Brown<sup>3</sup> reported 1,200 diastase units in the stools in four cases of carcinoma of the pancreas. The diagnosis in two of these cases was verified by operation.

TABLE 9.—VARIATIONS IN DIASTATIC ACTIVITY IN STOOLS FROM THE SAME PATIENT

Diastase Units in 1 Gm. Feces	Number of Exam- inations	Number of Cases	Diagnosis
0 to 20	4	2	Hepatic cyst; typhoid fever
0 to 70	6	2	Sprue; intestinal infantilism
7 to 400	9	4	Neurasthenia; chronic nephritis; chronic appendicitis
40 to 400	2	1	Scleritis
7 to 2,000	2	1	Suppurative sinusitis
400 to 2,500	4	2	Secondary syphilis; carcinoma of prostate
1,000 to 5,000	2	1	Neurasthenia

We have estimated the diastatic activity of the stools in the three cases of carcinoma of the pancreas previously discussed in the section on the urine in this disease. These patients came to operation or necropsy. They were all jaundiced. The feces contained large quantities of fat and a small amount of undigested muscle of fiber and starch granules. No diastatic activity was present in the feces of one patient. In a second case the stool contained 40 diastase units. Seven and no diastase units were present in two stools, collected on different days, from the third patient. At the necropsy in this case the duct of Wirsung was found to be completely occluded.

In our three cases of carcinoma of the pancreas and in the two reported by Wynhausen the diastatic activity in the feces is low. Nevertheless, the figures are not lower than those obtained by

Hirayama,<sup>27</sup> Friedmann,<sup>28</sup> and ourselves in patients without disease of the pancreas. Because of the method used by Brown, his figures do not admit of comparison with those obtained by other writers, but the values given are low.

#### THE FECES IN CHRONIC PANCREATITIS

Using Wohlgemuth's original method, Hirschberg<sup>15</sup> reported no diastase and 150 units in two stools in a case of chronic interstitial pancreatitis which came to necropsy. The case of Ehrmann and Kruspe,<sup>16</sup> discussed earlier in this paper, contained "repeatedly small amounts of ferment, but later frequently normal or increased content of the diastase." After a return to "normal" diastatic activity the azotorrhea persisted. No figures for diastatic activity are given. Brown<sup>8</sup> estimated the diastase activity of the stools in six cases of "chronic pancreatitis." The diagnoses were all verified by operation; "a small hard, diseased organ being found in each case." The diastase units in each case were 8,000, 7,500, 12,000, 8,000, 7,500 and 3,300 units. These figures cannot be compared with those of other writers, because of the difference in the method used. The values are low, however, since Brown considers 60,000 units the minimum normal limit. The diastatic activity in the feces of all these reported cases of chronic pancreatitis is low. But it is not lower than has been found in cases without pancreatic disease. The feces of forty-one, or 11.7 per cent. of these patients, contained less than 200 diastase units.

#### THE FECES IN ACUTE PANCREATITIS

Using Wohlgemuth's original method, Hirschberg<sup>15</sup> estimated the diastatic activity in the stools in two cases of acute pancreatic necrosis. During the third week after operation the feces in one case contained 85 units and "during convalescence" 185 units. The stools in the second case contained from none to 46.5 units during the first week following the operation. On the seventh day a stool resulting from castor oil contained 1,000 units. When the stools "became of normal consistency, from 200 to 250 diastase units were found." Wynhausen<sup>2</sup> reported thirty units in the stools in two cases of acute hemorrhagic pancreatitis. It is not stated that the diagnoses in these two cases were confirmed by necropsy or operation. Lindemann<sup>19</sup> made numerous estimations of the diastatic activity in the feces in two cases of acute pancreatic necrosis and in a case of a "retroperitoneal abscess reaching up to the pancreas." In the first case of acute necrosis the stools contained from 70 to 28.7 diastase units in the period between the second and nineteenth day following the operation. From the fifth to the seventh week the figures range from 532 to 287.5 units. Nine days after operation the stools in the second case contained 100 diastase

units. On the twelfth day following the operation a slough of pancreatic tissue was expelled through the operation wound. During the next week from 1,000 to 660 diastase units were present in the feces. The stools in the case of a "retroperitoneal abscess" contained five days prior to operation, 99.5 diastase units; three days prior, 237 units, and the day before, 250 units. Lindemann assumed that the pancreatic duct was occluded by pressure of the abscess. At operation an ileac fecal fistula was made. The fistulous discharge contained from 2,286 to 5,375 units. In one case Lindemann<sup>10</sup> made the diagnosis of pancreolithiasis. The diagnosis was not confirmed by operation or necropsy. The stools in this case contained from 8 to 17 units during the first five weeks of the illness; 625 to 1,000 units were present in the stools during the two succeeding two weeks. Crohn<sup>32</sup> found no diastatic activity in the duodenal contents or stool in a case of acute pancreatitis.

We have estimated the diastatic activity of the stools in Cases 1 and 4 of acute pancreatitis. These cases were discussed in the section on the urine in acute pancreatitis. The stools were liquid in character. Otherwise they were macroscopically and microscopically normal. No diastatic activity was present in the stools in the case of acute pancreatic necrosis two and four weeks after operation; 10 diastase units were found in the stool in the case of acute hemorrhagic pancreatitis nine days after operation.

In nine cases of acute pancreatitis the diastatic activity of the feces has been estimated. Low values were found in all. In a case of Lindemann's<sup>10</sup> and one of Hirschberg's<sup>10</sup> the diastatic activity increased during convalescence. The same was true in Lindemann's case of pancreolithiasis.

#### SUMMARY OF THE CLINICAL STUDIES ON THE FECES

The diastatic activity of the feces is reported in twenty-three cases of pancreatic disease in which the diagnoses were verified by operation or necropsy. In three additional cases the diagnoses were not confirmed. These twenty-six cases include those collected from the literature and the ones reported by ourselves. The values given for diastatic activity cannot be compared on a numerical basis because of the differences in the methods used. But they are all low. The diastatic activity although low, does not differ from that found in the stools in patients without disease of the pancreas reported by us or reported in the literature. The stools of forty-one persons, or 11.7 per cent. of those without disease of the pancreas, collected from the literature, contained from zero to 200 diastase units. Thirty-two, or 51.6 per

32. Crohn: The Functional Activity of the Pancreas, *Am. Jour. Med. Sc.*, 1913, **145**, 393.



cent., of our cases showed from none to 50 diastase units in the feces. The diastatic activity remained low in but four of these cases in which more than one stool was examined. The finding of low values in such a large percentage of persons with normal pancreas detracts greatly from the value of the estimation of the diastatic activity of the feces as an aid in the diagnosis of diseases of the pancreas. No one has made a large number of estimations in cases without pancreatic disease in which there was a greatly diminished or absent diastatic activity of the feces. It is quite possible that at times high values for the diastatic power would be found in such cases. This is made probable by the great fluctuation in the diastatic activity found in the stools in thirteen of our cases (Table 9). Lindemann<sup>19</sup> found similar variations in the stools of seven persons without pancreatic disease. A large number of estimations of diastatic activity have been made in the stools in five cases of pancreatic disease. In two cases of acute pancreatitis reported by Lindemann, persistently low values were found. In three other cases variable figures are reported. These three cases are discussed in the section on the factors that influence diastatic activity.

#### THE FECES IN EXPERIMENTAL PANCREATIC LESIONS

Wynhausen cut and tied the "pancreatic ducts" in a dog and partially separated the pancreas from the duodenal wall. After the operation there were "large amounts of diastase in the feces (500 and more units)." At a second operation three weeks later "part" of the pancreas was completely normal and "part" showed a marked "pancreatitis." A patent duct communicating with the duodenum was found. This was tied and cut. A considerable part of the pancreas was extirpated and the remaining portion was cauterized with the Paquelin cautery. The dog developed glycosuria and lost weight. Two days after this second operation the feces contained 500 diastase units, one week later 10 units, and on subsequent examinations, 12.5 and 5 units.

Wohlgemuth makes the statement that no diastatic activity is present in the feces of dogs in which the pancreatic ducts are occluded, while the feces of normal dogs contain a high diastatic activity. He gives no figures or protocols and the number of dogs examined is not stated.

We have studied the diastatic activity in the feces of three normal dogs and, also, in two of these animals after excluding pancreatic secretion from the duodenum. One of the dogs (Table 4) showed 7 diastase units in the stools before exclusion of pancreatic juice from the intestines, while six days after the operation 10 units were present. The dog passed stools in the intervening period, but they were lost in the urine. On the same day that the last stool examination was made

the dog was killed. No diastatic activity was present in the duodenal contents collected at the necropsy.

In Dog 2 (Table 5), 10 and 40 diastase units were present in the stools prior to the operation in which ligatures were placed around a portion of the pancreas. Pancreatic juice was not excluded from the intestines. On the fifth day following the operation the stools contained 10 diastase units.

In another of our dogs 200 diastase units were present in the stools nine days before the operation. The pancreas was completely separated from the duodenal wall and all of the gland except the processus uncinatus extirpated. No diastatic activity was found in the feces of this dog on the sixth day following the operation. The contents of the duodenum and jejunum removed at the necropsy of this animal showed no diastatic activity.

From these findings the conclusion is justified that exclusion of pancreatic secretion from the intestines in dogs is followed by a marked diminution, or by an absence, of the diastatic activity in the stools.

#### FACTORS INFLUENCING THE DIASTATIC ACTIVITY OF THE FECES

That a lack of pancreatic secretion is not the only cause of a low diastatic activity in the feces is suggested by the following observations:

1. The diastatic activity may be low or absent when the pancreas is normal. The stools in 11.7 per cent. of the patients with normal pancreas collected from the literature contained less than 200 diastase units; nineteen showed no diastase and seven less than 50 units. In nine of our sixty-eight cases with normal pancreas, the stools contained no diastase, and in fifteen the number of units was 10 or less.

2. Great variations occur in different stools from the same patient without any change in diet or in the patient's apparent condition. This is shown in Table 9.

3. The diastatic activity in the contents of the small intestine may be high and yet that of the stools low. In Lindemann's case of "retro-peritoneal abscess" the intestinal contents discharged from the fistula contained from 2,286 to 5,375 diastase units, while during the week prior to operation from 99.5 to 250 units were present in the feces. Werzberg<sup>33</sup> examined the diastatic activity of the intestinal contents in a case of carcinoma of the pylorus. At necropsy the pancreas was found to be normal. The contents of the duodenum and of the small intestines contained 1,533 diastase units, while the contents of the colon showed but 100 units.

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33. Werzberg: Zur Diagnostik der Pankreaserkrankungen, Arch. f. Verdauungskr., 1911, **17**, 533.



Variations in the absorption of pancreatic juice from the intestines would influence the quantity of diastase in the feces. Boldyreff<sup>34</sup> has shown that pancreatic secretion is normally absorbed from the intestines in dogs. Variations in the secretion of pancreatic diastase could produce corresponding differences in the diastase content of the feces. Crohn<sup>32</sup> states that the diastatic activity of the feces roughly corresponds to that of the duodenal contents. Both Crohn<sup>32</sup> and Holsti<sup>35</sup> found that the diastatic activity of different specimens of pancreatic juice varied widely. Crohn obtained pancreatic secretion by means of the duodenal tube, while Holsti made observations on a young man with a traumatic fistula. We have studied the effect of bacteria on the diastatic activity of eight stools from different persons. Portions of each stool were incubated at 38 C., refrigerated, and kept at room temperature for three days. Estimations of the diastatic activity were made at the beginning of the experiment and at the end of twenty-four, forty-eight, and seventy-two hours. The different portions of the same stools showed equal degrees of diastatic activity regardless of whether they were incubated, refrigerated, or kept at room temperature.

Strasburger<sup>36</sup> investigated the effect of different foods on the diastatic activity in the stools. His experiments were carried out on three persons without pancreatic disease. He concludes that the kind of food eaten has no effect on the diastatic power of the feces. Corbett<sup>11</sup> states that "the ordinary variations in hospital food from milk to full diet does not appear to have much influence on the output of diastase." Hawk<sup>37</sup> found that water drinking increased the diastatic power of the stools.

Strasburger<sup>36</sup> reported a large diastatic activity in the diarrheal stools from five patients. A small amount was found in the stools in two cases of constipation. From these findings he concludes that diarrhea causes an increase and constipation a decrease in the diastatic activity of the feces. We found in the stools of seven constipated persons with normal pancreas, no diastatic activity in five, 25 units in one and 3 units in one. In the case with 3 units two liquid stools resulting from magnesium sulphate each contained 3 units. Hirayama<sup>27</sup> reported from zero to 50 units in the stools of nine patients with diarrhea. We found from zero to 12 units in the diarrheal stools of seven patients

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34. Boldyreff, W.: Einige neue Seiten der Tätigkeit des Pankreas, *Ergebn. d. Physiol.*, 1911, **11**, 121.

35. Holsti: Beiträge zur Kenntnis der Pankreasssekretion beim Menschen, *Deutsch. Arch. f. klin. Med.*, 1913, **3**, 48.

36. Strasburger: Weitere Untersuchungen über Faecesgaehrung nebst allgemeinen Bemerkungen über das diastatische Ferment im menschlichen Stuhle, *Deutsch. Arch. f. klin. Med.*, 1900, **67**, 238.

37. Hawk, P. B.: The Activity of the Pancreatic Function Under the Influence of Copious and Moderate Water Drinking with Meals, *THE ARCHIVES INT. MED.*, 1911, **8**, 382 and 552.



and 400 units in another. These findings strongly suggest that the absence or diminution of diastase in the feces is often the result of absorption of the pancreatic secretion. In constipation, as is well known, absorption of the digested food is unusually complete, and this explains the absence of diastase. Bacterial action, as we have shown, does not destroy diastase. In some cases of diarrhea the absorption from the liquid intestinal contents may be good, as metabolism studies may show a normal absorption of nitrogen, carbohydrates, and fat even when the peristalsis is very active. Hence, the absence of diastase found in diarrhea is probably due to its absorption and not to its destruction.

Alkalies, neutral salts and acids influence the action of ferments.<sup>38</sup> The effect of these substances on the diastatic activity of the feces has been studied. Hawk<sup>37</sup> states that the accelerating effect of acids is without importance in clinical studies. Rotky<sup>30</sup> studied the effect of different concentrations of sodium chlorid on the diastatic activity of the dried feces. Different concentrations of salt mixed with the same stool produce variations ranging from zero to 427 units. Ury<sup>30</sup> studied the diastatic activity in the dried stools of one normal person. He claimed that senna increased, and magnesium sulphate decreased, the diastatic activity of the feces. But many of the highest values found by us were present in stools resulting from the administration of magnesium sulphate.

The chief cause of a diminished diastatic activity of the feces, and the only one that is clearly understood, is an absence of pancreatic juice or a diminished amount in the intestine. This would occur when the pancreatic ducts are occluded or the pancreatic tissue extensively diseased. If the duct of Wirsung alone were closed, pancreatic secretion would enter the duodenum through the accessory ducts. Nevertheless, the reported cases of obstruction of the duct of Wirsung have been accompanied by a low diastatic activity in the feces.

A low or absent diastatic activity of the feces is not conclusive evidence of a diminution or a lack of pancreatic secretion entering the intestines, as it is found in many cases without pancreatic disease. Lindemann<sup>19</sup> considers that for diastase determinations to be of value in diagnosing disease of the pancreas there must be a high diastase content in the urine and a low content in the feces. This occurred in three of our cases with normal pancreas.

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38. Oppenheimer, C.: *Die Fermente und ihre Wirkungen*, Leipzig, 1909, Ed. 3, p. 102.

39. Ury: *Ueber den quantitativen Nachweis von Fermenten in den Faeces*, *Biochem. Ztschr.*, 1909-1910, **23**, 143.

## CONCLUSIONS

1. The estimation of the diastatic activity of the urine is of very slight value in the diagnosis of pancreatic disease. In rare instances, however, a large amount of diastase is present—more than 500 units per cubic centimeter. This finding strongly suggests disease of the pancreas.

2. The diastatic activity in the urines of persons with normal pancreas varies widely.

3. Injury to the pancreas of dogs may be followed by a marked increase in the diastatic activity of the urine, but this does not always occur.

4. Ether anesthesia may increase the diastase in the urine of dogs.

5. The estimation of diastatic activity in the feces is of slight value as an aid to the diagnosis of disease of the pancreas.

6. Obstruction of the pancreatic ducts in man results in a low diastatic activity in the feces.

7. The diastatic activity in the feces of persons without pancreatic disease varies widely.

8. The absence of diastatic activity in constipation and in other conditions, in which the pancreas is normal, is probably due to complete absorption of the diastase by the intestines.

9. Exclusion of pancreatic secretion from the intestines in dogs is followed by a marked diminution or absence of diastatic activity in the stools.

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## THE SUPRARENAL SYSTEM AND CARBOHYDRATE METABOLISM \*

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Prior to the experiments of Blum<sup>1</sup> in 1901, when he demonstrated that injections of a watery extract of the adrenal glands constantly give rise to glycosuria in dogs, very little attention had been given to the action of the suprarenal secretion on metabolism. Claude Bernard's<sup>2</sup> conception of an internal secretion enunciated in 1855, and the publication, in the same year, of Thomas Addison's book containing his observations on the association of a definite clinical syndrome with pathologic changes in the adrenal glands, prepared the way for Brown-Séquard's<sup>3</sup> well known experiments. His conclusions, though in part erroneous, were epoch-making. Because of the stimulus it supplied to investigations in the new field of internal secretion, the value of this work was tremendous. But so far as the adrenals were concerned, these early researches had been confined chiefly to studies of their effect on blood pressure, local changes in the tissues and blood vessels at the site of injection, and to general symptoms following extirpation experiments.

Abundant experimental data, and much speculation as to the rôle of the adrenal secretion in carbohydrate metabolism has accumulated in the fifteen years since Blum's observation. This work has, for the most part, represented efforts to discover the mechanism by which the suprarenal system, and more particularly the adrenal glands themselves, intervene in sugar metabolism, with the idea constantly present that they may be implicated in the perversions of metabolism in diabetes mellitus.

In addition, however, to the glycosuric effects of epinephrin injections, there are other observations which indicate that the adrenals affect the metabolism of carbohydrates. Porges<sup>4</sup> and later Bernstein<sup>5</sup> reported a lowering of blood sugar in Addison's disease, while Fuchs

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1. Blum: *Deutsch. Arch. f. klin. Med.*, 1901, **71**, 146; and *Arch. f. d. ges. Physiol. (Pflüger's)*, 1902, **90**, 628.

2. Bernard, Claude: *Leçons de physiologie expérimentelle au Collège de France*, Paris, 1855.

3. Brown-Séquard: *Compt. rend. Soc. de biol.*, 1889, pp. 415, 420, 430, 451.

4. Porges: *Ztschr. f. klin. Med.*, 1909, **69**, 341.

5. Bernstein: *Berl. klin. Wchnschr.*, 1911, **48**, 1874.



and Roth<sup>6</sup> found, after injections of epinephrin, a distinct rise in the respiratory quotient in patients with this disease. Porges<sup>7</sup> also showed that the blood sugar in adrenalectomized dogs regularly falls during the time that the animal survives. The experiments of Bierry and Mallozel<sup>8</sup> confirmed this observation. They found that the concentration of sugar in the blood, after extirpation of the adrenals, fell to one fifth or one half of the original amount and remained there. They also showed that these animals were more resistant to epinephrin injections than normal animals so far as the production of hyperglycemia is concerned. More will be said about these observations later on in discussing the phase of carbohydrate metabolism affected by the adrenal secretion. It is obvious, however, from what has been said, that the adrenal glands by means of their internal secretion, and in particular by the active principle of the medullary portion, play some part in carbohydrate metabolism. The possibility that this power to alter and in part control the metabolism of sugar bears some relation to the pathogenesis of diabetes mellitus is, of course, of the greatest importance, and one which has formed the chief stimulus to the investigations. With this possibility in mind we shall review the experimental work and hypotheses which have been offered to explain the mechanism by which the secretion of the adrenals affects the storage and combustion of carbohydrates.

But before entering on a consideration of this question it will be well to recall briefly the important facts in normal carbohydrate metabolism. Carbohydrate food enters the body by the alimentary tract in the form of sugars and starches. By the action of diastatic enzymes of the saliva and pancreatic secretion the latter are split into sugars. In the intestine, chiefly in the upper portion of the small intestine, the sugars are acted on by the enzymes of the intestinal mucosa, namely, invertase, maltase and lactase, and thereby converted into one or other of the monosaccharids. By far the largest portion of the ingested carbohydrate is absorbed in the form of dextrose, but levulose and galactose have also been demonstrated in the portal blood. In the liver, glycogen is formed from these sugars and stored within the hepatic cells, probably by the action of an enzyme (glycogenase?) which is present in the liver cells. This formation of glycogen, which depends chiefly on glucose, but also to a slight extent on levulose and galactose, is called glycogenesis. Whenever the cells of the body, especially the striated muscles, require glucose for heat or energy needs, the glycogen stored in the liver is broken down by the glycogenase, and glucose is discharged into the hepatic veins, and thus to the general circulation.

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6. Fuchs and Roth: *Ztschr. f. exper. Path. u. Therap.*, 1912, **10**, 187.

7. Porges: See Note 4.

8. Bierry and Mallozel: *Compt. rend. Soc. de biol.*, 1908, **60**, 232.

This process by which glucose is formed from glycogen is termed glycogenolysis. The tissues of the body either burn the glucose thus brought to them by the blood stream, forming carbon dioxide and water as the end-products, or they store it as glycogen. So accurately adjusted is this mechanism by which the liver supplies the circulating blood, and, indirectly, the tissues, with glucose, according to the tissue requirements, that the concentration of the glucose of the blood remains practically constant, despite great variations in the quantities the tissues use. Normal well-fed dogs made to run on the treadmill almost to the point of exhaustion show little, if any, alteration in the glucose concentration of the blood, despite the enormous consumption by the muscular tissues entailed during such exercise. Under physiologic conditions the blood sugar is kept between 70 and 100 mg. per 100 c.c. of blood. But in order that these processes shall proceed in this way it is essential that the islands of Langerhans be functioning. Removal of the pancreas promptly upsets this mechanism; the blood sugar rises, glycosuria appears, the glycogen stores become impoverished, and the animal presents an essentially complete picture of diabetes mellitus.

Any disturbance in this mechanism for sugar control is promptly shown by changes in sugar concentration of the circulating blood. Overproduction by the liver, that is, hyperglycogenolysis, is at once followed by a rise in the sugar of the blood. Diminished or arrested consumption, that is, a disturbance in glycolysis, produces a similar result.

Following an injection of epinephrin there occurs some disturbance in the physiologic sequence of events just outlined, a disturbance manifested by the appearance of considerable quantities of sugar in the urine. One or more of the several phases of carbohydrate metabolism must, therefore, be affected either in rate or character. We must consider each of the possibilities: does epinephrin alter the process of sugar absorption from the intestine; or is it in the processes of formation or breaking down of glycogen that it intervenes; or does it affect the oxidation of the sugar; or does it act on the internal secretion of the pancreas, rendering it incapable of exerting its normal control of sugar metabolism; or, finally, does it simulate the action of phlorizin and lower the threshold for sugar excretion by the kidneys?

There is, perhaps, one further possibility which, alone, could hardly account for the epinephrin effects, but as an ancillary factor, might play a part. This is glycogenesis, or the formation of glucose from protein. If epinephrin caused the breaking down of the protein molecule and the liberation of the carbohydrate fraction, this could increase the effects of such a carbohydrate disturbance as epinephrin produces; but with glycogenic, glycogenolytic and glycolytic processes proceeding



normally, glyconeogenesis could not explain the metabolic changes following an increase of the circulating epinephrin. Such an increased production of carbohydrate would be quite comparable to carbohydrate feeding in a normal animal—the excess would either be stored as glycogen or oxidized.

It is not necessary to discuss the possibility that epinephrin affects the process of sugar absorption by the intestines; suffice it to say that there is neither theoretical reason nor experimental evidence pointing to any such action.

We shall, therefore, proceed to examine the experimental data and the conclusions which may be drawn from them in so far as they throw light on the remaining theoretically possible explanations of the mechanism of the epinephrin action.

Blum's results were promptly confirmed (Zuelzer,<sup>9</sup> Metzger,<sup>10</sup> Herter and Richards,<sup>11</sup> Paton,<sup>12</sup> Lazarus<sup>13</sup>), and it soon became well established that the injection of 1.0 mg. of epinephrin per kilo of body weight is followed by a glycosuria which appears in one half to two hours, and may last a few hours to a day or longer. Moreover, it does not matter whether a saline extract of the fresh gland be employed or one of the pure products, such as the adrenalin chlorid (Aldrich,<sup>14</sup> Takamini<sup>15</sup>), the epinephrin of Abel,<sup>16</sup> adrenin, or the synthetic product.<sup>17</sup>

First Zuelzer<sup>9</sup> and then Metzger<sup>10</sup> and Paton<sup>12</sup> showed that the glycosuria is accompanied by an hyperglycemia, thus ruling out the possibility that the mechanism is similar to that of phlorizin diabetes, in which, despite glycosuria, there is hypoglycemia, and probably a lowering of the threshold for sugar excretion by the kidney. Vosburg and Richards<sup>18</sup> obtained, after epinephrin injections, a more marked hyperglycemia in well fed animals than in those which had been starved, suggesting thus that the glycogen stores had been called on to give up glucose to the blood. Pollak<sup>19</sup> showed that both hyperglycemia and glycosuria are more readily produced by subcutaneous than by intravenous or intraperitoneal administration. He explains this by the assumption that the carbohydrate mechanism is more sensitive to epinephrin than the vasoconstrictors, and that the slow absorption after subcutaneous injections allows the epinephrin concentration of the blood to rise only to a level at which the carbohydrate mechanism is

9. Zuelzer: Berl. klin. Wchnschr., 1901, **38**, 1209.

10. Metzger: München. med. Wchnschr., 1902, **12**, 478.

11. Herter and Richards: Med. News, New York, 1902, **80**, 201.

12. Paton: Jour. Physiol., 1903, **29**, 286.

13. Lazarus: Cited by Shafer, Brit. Med. Jour., 1908, **1**, 1277.

14. Aldrich: Am. Jour. Physiol., 1901, **5**, 457.

15. Takamini: Am. Jour. Pharm., 1901, **11**, 523.

16. Abel: (Hoppe-Seyler's) Ztschr. f. Physiol. Chem., 1899, **28**, 318.

17. Amberg: Arch. Intern. de Pharmacol., 1902, **11**, 57.

18. Vosburg and Richards: Am. Jour. Physiol., 1903, **9**, 35.

19. Pollak: Arch. f. exper. Path. u. Pharmacol., 1909, **61**, 149.



affected, while the more rapid entrance of the epinephrin into the circulation after intravenous or intraperitoneal injection raises the concentration in the blood sufficiently high to cause vasoconstriction. Consequently a diminished amount of blood passes through the liver, and thus less glucose is mobilized. Straub<sup>20</sup> and Ritzmann,<sup>21</sup> on the basis of infusion experiments, have shown that the glycosuria runs almost parallel with the epinephrin concentration of the blood, and Ritzmann

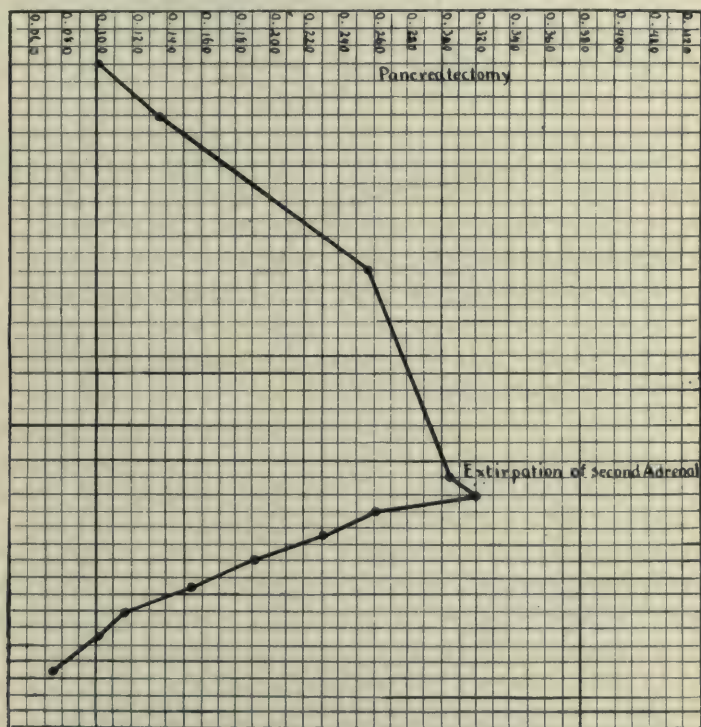


CHART 1.—Blood sugar curve in depancreatized dog, with extirpation of adrenals. Ordinates = per cent. reducing substance in blood. Abscissa = time: Each square = two hours.

puts forward the idea that epinephrin acts to maintain, by sympathetic stimulation, a *Zuckertonus* as well as a *Gefässtonus*. Underhill's<sup>22</sup> experiments with epinephrin infusions corroborate Ritzmann's results. Satisfactory investigations as to the parallelism between sugar and epinephrin concentrations in the blood are lacking.

Of considerable importance in determining how and where epinephrin acts is, first, the question of the effect of epinephrin injections on the glycogen content of the liver, and, second, whether or not it is

20. Straub: München. med. Wchnschr., 1909, **56**, 493.

21. Ritzmann: Arch. f. exper. Path. u. Pharmacol., 1909, **61**, 231.

22. Underhill: Jour. Biol. Chem., 1911, **9**, 13.

still capable of causing hyperglycemia and glycosuria after the glycogen depots of the body have been exhausted.

The first of these questions has been definitely settled. Doyon and Kareff,<sup>23</sup> Gatin-Gruzewska,<sup>24</sup> Agadschanianz<sup>25</sup> and Pollak<sup>26</sup> have shown that epinephrin diminishes the quantity of glycogen in the liver. Schirokauer and Wilenko<sup>27</sup> went a step further and showed that the increased glycogenolysis in animals treated with epinephrin is not dependent on an increased production of liver diastase. Furthermore, Gatin-Gruzewska<sup>24</sup> and Agadschanianz<sup>25</sup> demonstrated that the muscles also lose glycogen. So far as I am aware there are no trustworthy experiments contradicting this conclusion.

If the second of these questions could be answered affirmatively, it would constitute a strong support for the view that epinephrin acts not merely on the liver, causing it to discharge glycogen, but also that it is capable of raising the concentration of the blood sugar, either by interfering with the glycolytic processes in the tissues or by inducing glyconeogenesis, or by inhibiting the pancreas. It becomes necessary, therefore, to examine more closely the evidence on which conclusions have been based as to the effect of epinephrin on animals whose livers have been rendered glycogen-free.

Blum,<sup>1</sup> in his early communications, asserted that epinephrin glycosuria occurred even when the glycogen stores had been exhausted before the injection, but his conclusion was based on the assumption that starvation alone will remove all the glycogen from the liver. This is open to question, because Pflüger<sup>28</sup> has shown that a dog after starving twenty-eight days still contains enough glycogen to form 100 gm. of sugar. Ringer<sup>29</sup> found no increase in glycosuria following epinephrin injections after the liver had been rendered glycogen-free by phlorizin. This, however, is not convincing proof, because little is known of the action of phlorizin, beyond the fact that it lowers the threshold for sugar excretion by the kidney. It may also interfere with the mechanism involved in epinephrin glycosuria. Paton<sup>12</sup> and Herter and Richards<sup>11</sup> offered evidence in support of Blum. They found that dogs which had been either fasted or fed on a diet of material from which no glycogen could be formed and then phlorizined, still responded with glycosuria to epinephrin injections. It seems doubtful, however, that the animals were glycogen-free before the injection. Frank and Isaac<sup>30</sup> found that after dogs were poisoned with phos-

23. Doyon and Kareff: *Compt. rend. Soc. de biol.*, 1904, **56**, 66.

24. Gatin-Gruzewska: *Compt. rend. Acad. de sc.*, **142**, 1165.

25. Agadschanianz: *Biochem. Ztschr.*, 1907, **2**, 148.

26. Pollak: *Arch. f. exper. Path. u. Pharmacol.*, 1909, **61**, 153.

27. Schirokauer and Wilenko: *Ztschr. f. klin. med.*, 1910, **70**, 257.

28. Pflüger: *Arch. f. d. ges. Physiol.*, 1902, **91**, 119.

29. Ringer: *Jour. exper. Med.*, 1910, **12**, 105.

30. Frank and Isaac: *Ztschr. f. exper. Path. u. Therap.*, 1910, **7**, 326.



phorus no hyperglycemia followed epinephrin injections. Phosphorus is known to cause a disappearance of the liver glycogen, but the absence of epinephrin glycosuria during such a profound metabolic disorder as phosphorus causes does not settle the question. Velich<sup>31</sup> found no epinephrin glycosuria after extirpation of the liver in frogs. Falta and

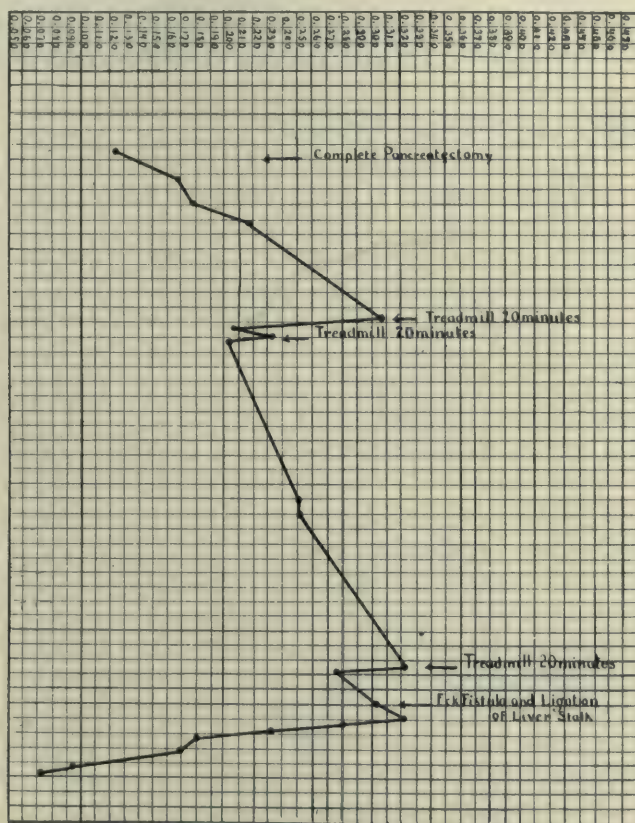


CHART 2.—Blood sugar curve in depancreatized dog with Eck fistula and ligation of liver stalk. (The first portion of this curve was made for another experiment in which the effect of exercise on the blood sugar was being studied.) The rapid and uninterrupted drop in the amount of reducing substance in the blood after the liver was excluded from the circulation is very striking. Ordinates represent per cent. of reducing substance in the blood. Abscissa = time: Each square = two hours.

Priestly,<sup>32</sup> using rabbits, tied off the liver vessels and found that, despite epinephrin injections, the blood sugar fell to 0.04 per cent. The conclusion from this seems to be that the liver glycogen is essential for the maintenance of the blood sugar level, and that the glycogen stores

31. Velich: Virchows Arch. f. path. Anat., 1906, **184**, 345.

32. Falta and Priestley: Berl. klin. Wchnschr., 1911, **48**, 2102.



in the muscles are inadequate for this. Michaud's<sup>33</sup> experiments on dogs with Eck's fistulas are important. In only one out of six dogs did epinephrin cause a rise in blood sugar. His animals remained in good condition after the operative interference, a condition which has usually been lacking in experiments aimed to settle this point, and one which has doubtless contributed to the contradiction of results. Pollak<sup>34</sup> induced convulsions with strychnin in starved rabbits, presumably glycogen-free. Although it did not produce hyperglycemia, epinephrin did cause a disturbance in carbohydrate metabolism, shown by the deposition of glycogen in the liver.

It is not easy to give a definite interpretation of the experimental data mentioned; experiments of many types have been performed to determine whether the glycosuric and hyperglycemic action of adrenal persists without available glycogen in the liver, but, as the above mentioned investigations show, there has been by no means a uniformity in the results. Perhaps the experiments of Pollak<sup>29</sup> and Michaud<sup>33</sup> are the most significant; from them we may conclude, with some reserve, however, that an available supply of glycogen in the liver is essential for the production of hyperglycemia by epinephrin injections, and, furthermore, that an excess of epinephrin in the blood stimulates the formation of glycogen in a glycogen-free liver. But there are other reasons, besides those advanced by Pollak, which lead us to believe that epinephrin has some influence on glycogen formation, as well as on glycogenolysis. Schwarz,<sup>35</sup> using rats, because they survive adrenalectomy for a long time, determined the glycogen content (Pflüger's method) after both adrenals had been extirpated, and found only mere traces or complete absence of glycogen. He concludes that after adrenalectomy the power to store glycogen is lost. This work has been confirmed by Kahn and Starkenstein,<sup>36</sup> who not only demonstrated a loss of liver glycogen following adrenalectomy, but demonstrated also that such animals acquire an unusual tolerance for epinephrin.

I have administered large quantities of glucose intravenously and by the alimentary tract to adrenalectomized dogs, and have found that when, by thus saturating the organism with sugar, the concentration of sugar in the blood has been raised to 560 mg. per 100 c.c., little or no glycogen is demonstrable in the liver cells when stained by Best's method. Control animals which have been starved, but not adrenalectomized, receiving the same amounts of sugar per kilo body weight, show abundant glycogen granules in the liver cells. Since we know that an excess of epinephrin in the blood causes a discharge of gly-

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33. Michaud: *Verhandl. d. deutsch. Kongr. f. inn. Med.*, Wiesbaden, 1911, p. 561.

34. Pollak: *Arch. exper. Path. u. Pharmakol.*, 1909, **61**, 166.

35. Schwarz: *Arch. d. ges. Physiol. (Pflüger's)*, 1910, **134**, 259.

36. Kahn and Starkenstein: *Arch. f. d. ges. Physiol. (Pflüger's)*, 1911, **139**, 181.

cogen from the liver, the observation that a diminished amount of epinephrin in the blood is associated with an absence of glycogen, carries with it the conclusion that epinephrin performs a double function, so far as glycogen metabolism in the liver is concerned. It is necessary for glycogenesis, and when present in excess it stimulates glycogenolysis. Or, stated in another way, when it is absent, glycogen storage is inhibited; when it is present in excess, the transformation of glycogen to glucose is accelerated.

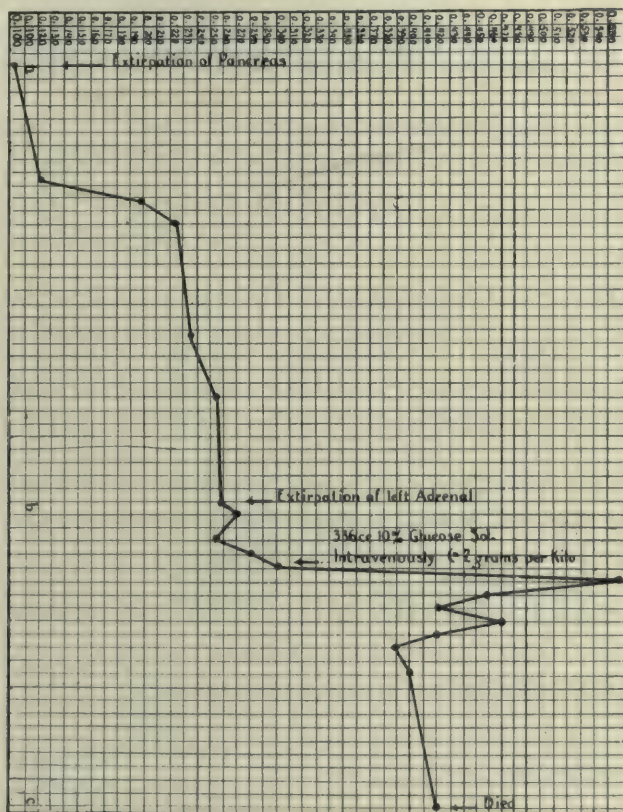


CHART 3.—Blood sugar curve in depancreatized dog after extirpation of the adrenals, followed by intravenous injection of glucose solution—2 gm. per kilo. Ordinates = per cent. of reducing substance in blood. Abscissa = time: a-b each square = two hours; b-c each square = one-half hour.

Having now satisfactory evidence that the internal secretion of the adrenals intervenes in carbohydrate metabolism in these two ways, we must proceed to examine the data which throw light on the chemical or physical processes by which these functions are performed. We can imagine that such a mechanism is dependent on direct hormone action within the liver cells, the epinephrin acting, on the one hand, on the glucose brought by the portal blood, converting it to glycogen, and, on



the other hand, on the glycogen of the liver, converting it to glucose. Or we can think of it as producing its effects by controlling the blood supply of the liver, the changes in the condition of the carbohydrate in the liver being then dependent on the constriction or dilatation of the blood vessels. We may also, as some investigators hold, believe that its action depends on a specific power to affect the internal secretion of the pancreas. Finally, if it could be shown that glycogenesis and glycogenolysis are controlled by nervous impulses carried by the sympathetic, the well known stimulating effects of epinephrin on sympathetic nerve endings might conceivably explain the mechanism.

That there is an intimate relation between the nervous system and the sugar content of the blood has long been well known. Sixty-one years ago Claude Bernard<sup>2</sup> published the results of his observations on the appearance of glycosuria following puncture of the floor of the fourth ventricle. Many investigators have been interested since then in studying experimentally the mechanism by which piqûre glycosuria is brought about.

Naunyn<sup>37</sup> found a hyperglycemia of 0.81 per cent. four hours after piqûre. Because of the inexactness of the methods of blood sugar determination, these early observations are open to question. Bang, Ljungdahl and Bohm,<sup>38</sup> however, found a blood sugar of 0.38 per cent. after piqûre in rabbits, and this has been confirmed by others. Eckhardt<sup>39</sup> and later Pflüger<sup>40</sup> agreed with the opinion of Bernard that the stimuli produced by piqûre are carried directly to the liver by way of the splanchnics. Blum's<sup>1</sup> discovery of epinephrin glycosuria, however, suggested at once that epinephrin might be a factor in piqûre glycosuria. That the secretion of epinephrin is under the control of the sympathetic has been well established (Asher,<sup>41</sup> Popielski,<sup>42</sup> Tscheboksareff<sup>43</sup>). Pollak<sup>44</sup> showed that piqûre and other centrally acting causes of hyperglycemia are ineffective if the splanchnics are severed, and Macleod and Pearce,<sup>45</sup> by electric stimulation of the splanchnics, produced hyperglycemia. The results of Waterman and Smit,<sup>46</sup> who, using the Meltzer-Ehrmann reaction as a test for increase of epinephrin in the blood, claimed to have demonstrated an increase following piqûre, have met with some opposition. Later researches by Kahn,<sup>47</sup>

37. Naunyn: Arch. f. exper. Path. u. Pharmakol., 1874, **3**, 85.

38. Bang, Ljungdahl and Bohm: Beitr. z. chem. Physiol. u. Path. (Hofmeister), 1907, **10**, 1.

39. Eckhardt: Beitr. z. Anat. u. Physiol., 1869, **4**, 4, 138.

40. Pflüger: Das Glykogen, Ed. 2, 1905.

41. Asher: Centralbl. f. Physiol., 1910, **137**, 927.

42. Popielski: Arch. f. d. ges. Physiol., 1911, **139**, 571.

43. Tscheboksareff: Centralbl. f. Physiol., 1910, **137**, 137.

44. Pollak: Arch. f. exper. Path. u. Pharmakol., 1909, **61**, 376.

45. Macleod and Pearce: Am. Jour. Physiol., 1912, **29**, 419.

46. Waterman and Smit: Arch. f. d. ges. Physiol. (Pflüger's), 1908, **124**, 198.

47. Kahn: Arch. f. d. ges. Physiol. (Pflüger's), 1912, **144**, 251 and 396.



Lopez,<sup>48</sup> and v. Brucke<sup>49</sup> failed to confirm the findings of Waterman and Smit. It should be remembered that the tests for epinephrin in the blood are not as precise as could be desired<sup>50</sup> and the results reported should not be too readily accepted. There are four more or less reliable physiologic tests for the presence of minute quantities of epinephrin in the blood: (1) its mydriatic (dilatory) action on the pupil of the excised frog's eye; (2) its effect in raising the arterial blood pressure; (3) its power of lessening the rhythmic contractions of the longitudinal coat of the intestine immersed in Ringer's solution; (4) its stimulating action on the rhythmical contractions which occur in the excised uterus of the virgin rabbit in oxygenated Ringer's solution. The reason for caution in interpreting such results is that serum may contain substances which stimulate and substances which inhibit<sup>51</sup> the action of epinephrin. Borberg,<sup>52</sup> using an improved technic for the Meltzer-Ehrmann reaction, found a marked rise in the adrenalin content of the adrenal vein after diabetic puncture. Kahn,<sup>53</sup> moreover, on the basis of indirect evidence, believed that piqûre caused a discharge of epinephrin. He found a decrease of chromaffin substance in the adrenals after piqûre, and assumed from this that there was an increased secretion. Jarisch<sup>54</sup> was unable to confirm this. Trendelenburg and Fleischhauer<sup>55</sup> offered further indirect evidence against the view that piqûre is followed by an increased concentration of epinephrin in the blood. They determined the amount of epinephrin which, by continuous intravenous infusion, would produce a glycosuria equivalent to that following piqûre, and observed that when such amounts of epinephrin were in the blood there was at the same time a marked rise in the blood pressure. Since this is absent after piqûre, they believe that an hyper-adrenalinemia is not the essential factor in piqûre glycosuria.

Further evidence as to the rôle of the adrenals in the production of piqûre hyperglycemia and glycosuria is found in the numerous experiments on the effect of piqûre or splanchnic stimulation after extirpation of the adrenals. Mayer<sup>56</sup> did piqûre on twenty-five adrenalectomized rabbits and failed to produce glycosuria. Landau<sup>57</sup> confirmed this. Kahn<sup>58</sup> found that after extirpation of the adrenals rabbits showed no glycosuria. In later experiments,<sup>58</sup> however, he found

48. Lopez: Arch. f. d. ges. Physiol. (Pflüger's), 1912, **145**, 311.

49. Von Brucke: München. med. Wchnschr., 1911, p. 1389.

50. Macleod: Diabetes, London, 1913.

51. Stewart: Jour. Exper. Med., 1911, **14**, 377.

52. Borberg: Dissertation, Kopenhagen, 1912.

53. Kahn: Arch. f. d. ges. Physiol. (Pflüger's), 1911, **140**, 209.

54. Jarisch: Ztschr. f. exper. Path. u. Therap., 1913, **13**, 520.

55. Trendelenburg and Fleischhauer: Ztschr. f. d. ges. exper. Med., 1913, **1**, 369.

56. Mayer: Compt. rend. Soc. de biol., 1906, **60**, 1123.

57. Landau: Experimentelle Nebennieren-Studien, Dorpat, 1908.

58. Kahn: Arch. f. d. ges. Physiol. (Pflüger's), 1909, **128**, 519.

that piqûre causes a loss of chromaffin substance from the adrenals, and from this concluded that piqûre stimulates the secretion of epinephrin. Freund and Marchand<sup>59</sup> found that piqûre in adrenalectomized animals is followed by marked hyperglycemia, but they found no sugar in the urine. They concluded that its hyperglycemic action does not depend on the adrenals, but results from direct stimulation of the liver. Wertheimer and Battez<sup>60</sup> found that dogs and cats exhibit a difference in their reaction to piqûre. Adrenalectomized cats showed a marked glycosuria (3.63 to 17.5 gm.) after piqûre, but dogs on which they performed similar experiments, showed none. Gautrelet and Thomas<sup>61</sup> stimulated the splanchnics after extirpation of the adrenals and found no glycosuria.

That the blood sugar curves<sup>62</sup> after epinephrin injections and after piqûre are different affords some evidence against the view that the mechanism is similar, but the great variability of the curves after piqûre makes deductions from this hazardous.

Perhaps the most illuminating experiments dealing with this problem are those of Macleod<sup>63</sup> and his collaborators. Realizing that the disturbances of respiration and circulation following piqûre vitiate, in part at least, the hyperglycemic effects of this procedure, he demonstrated that the cava blood contains an increased quantity of reducing substance after stimulation of the great splanchnic nerve with the adrenals intact. After excision of the gland on the left side, stimulation of the nerve on the same side causes no rise in the blood sugar. Gautrelet and Thomas<sup>61</sup> had obtained results similar to these, but since this did not prove that the hyperglycemia was due to an increased secretion of epinephrin, Macleod went further and elaborated a type of experiment first done by Kaufmann.<sup>64</sup> He sectioned the nerve path between the adrenals and the liver, that is, the hepatic plexus. When this was done he found that stimulation of the left splanchnic was only rarely followed by an increase in the sugar of the blood, and that when this occurred it was less intense than usual. The conclusion from this is that it cannot be merely an increased epinephrin secretion which causes the hyperglycemia. He then applied electrical stimulation directly to the hepatic plexus and found that when the adrenals were intact such stimulation caused a striking hyperglycemia, whether the fibers central to the point of stimulation were cut or uncut. In the absence of the adrenal glands, however, stimulation of the hepatic plexus had no effect. The conclusion arrived at from these experi-

59. Freund and Marchand: *Arch. f. exper. Path. u. Pharmakol.*, 1914, **76**, 324.

60. Wertheimer and Battez: *Compt. rend. Soc. de biol.*, 1914, **86**, 617.

61. Gautrelet and Thomas: *Compt. rend. Soc. de biol.*, 1909, **67**, 233.

62. Bang: *Der Blutzucker*, Wiesbaden, 1913, p. 113.

63. Macleod: *Diabetes*, London, 1913, and Macleod and Pearce: *Am. Jour. Physiol.*, 1912, **29**, 419.

64. Kaufmann: *Arch. de Physiol.*, 1895, **27**, 266.



ments is that only when the adrenal glands are intact is it possible, by stimulation of the nerves supplying the liver, to excite hyperglycogenolysis and hyperglycemia. Some influence exercised by the adrenal glands is evidently essential for the functional integrity of the nerves which control the process of glycogenolysis. That this rôle of activating the receptive substance which, according to the experiments of Elliott<sup>65</sup> and others, is believed to lie between the nerve terminations and the cell substance, is not out of harmony with the fact that an excess of epinephrin in the blood causes an increase of glycogenolysis, is shown by other experiments of Macleod. Hyperglycemia was produced by injecting epinephrin into the portal vein after the sympathetic stimuli had been stopped by cutting the hepatic plexus. This explanation of the mechanism by which epinephrin produces its glycosuric effect has recently been substantiated by the work of Freund.<sup>66</sup> Taking advantage of the fact that rabbits have a collateral liver circulation through diaphragmatic vessels which is sufficient to prevent necrosis when the hepatic artery is tied, he cut the hepatic artery and hepatic plexus before doing piqûre. His results were not entirely constant, but on the whole indicated that piqûre does not cause hyperglycemia unless the nerve path between adrenals and liver is intact. Hence piqûre hyperglycemia is not dependent on an increase of epinephrin in the blood.

The possibility that the internal secretion of the adrenals interferes with the pancreatic control of carbohydrate metabolism by a specific inhibitory action, is an attractive theory, but one for which sound experimental proof is still lacking. In the first place it is not yet clear just what rôle in carbohydrate metabolism is played by the pancreas. The secretion which performs this function has never been isolated. The effects of removal of the gland are well known, but just how it acts when present, it is impossible to say. From the work of Hédon<sup>67</sup> and later that of Lombroso,<sup>68</sup> MacCallum<sup>69</sup> and Forsbach<sup>70</sup> it may be accepted as an established fact that the pancreas, independent of its external secretion, and of the nerve connections, performs an important function in the mobilization or utilization of glucose, and that probably this is accomplished by an internal secretion. Investigators are divided on the question of whether or not the hyperglycemia and glycosuria of pancreatic insufficiency are due to a primary underconsumption or a primary overproduction, and it is not necessary for our present purpose to discuss this question, except insofar as it

65. Elliott: Compare Swale Vincent: *Ergebn. der Physiol.*, 1910, **9**, 451.

66. Freund: *Arch. f. exper. Path. u. Pharmacol.*, 1914, **76**, 311.

67. Hédon: *Compt. rend. Soc. de biol.*, 1890.

68. Lombroso: *Ergebn. der Physiol.*, 1910, **9**, 1.

69. MacCallum: *Bull. Johns Hopkins Hosp.*, 1909, **20**, 265.

70. Forsbach: *Deutsch. med. Wchnschr.*, 1908, **34**, 910; *Arch. f. exper. Path. u. Pharmacol.*, 1908, **60**, 131.



involves the theory advanced by Eppinger, Falta and Rudinger<sup>71</sup> regarding the specific interaction of the adrenals and pancreas. They found that in depancreatized dogs injections of epinephrin caused an increase in the sugar excretion, and a rise of the D: N ratio, but the objection has been made that they offer no proof that the diabetes was complete before the epinephrin was injected. Ringer<sup>20</sup> failed to produce an increase in the glycosuria of fully phlorizinized dogs by epinephrin injections. Zuelzer's<sup>72</sup> observations that injections of pancreas extract rendered epinephrin nonglycosuric seemed to support the view that after pancreatectomy the diabetic manifestations appear because the internal secretion of the adrenal is then permitted, free from pancreas opposition, to act as a stimulus on the sugar output of the liver; but it was later shown that<sup>73</sup> many irritating substances when injected intraperitoneally have the same inhibitory effect on epinephrin glycosuria. Frugoni,<sup>74</sup> however, found that the inhibitory effect is obtained when the extract and epinephrin are injected subcutaneously. Loewi found that after removal of the pancreas the serum has an increased mydriatic power, and believes that this is due to the action of the epinephrin in the serum from which the inhibiting pancreatic secretion has been removed. He found human diabetic patients more susceptible to epinephrin mydriasis than normal controls.

Biedl and Offer<sup>75</sup> found that the lymph from the thoracic duct contains something which antagonizes the action of epinephrin on the excised frog's eye, and also inhibits epinephrin glycosuria. Believing this substance to be the internal secretion of the pancreas, they support the view of a mutually inhibitory action by the pancreas and adrenals.

A few experiments on the effect of adrenal extirpation on pancreatic diabetes in animals have been attempted. The difficulty here has been that the animals die so soon after removal of the adrenals that conclusions drawn from blood sugar determinations while the animals were in a moribund state are untrustworthy. Frouin<sup>76</sup> extirpated about three quarters of the adrenal substance from animals which had previously been rendered diabetic by removal of the pancreas. He found that the intensity of the diabetes was decreased as compared with controls. Mayer<sup>77</sup> destroyed by cauterization the adrenals of depancreatized dogs, but the animals survived the operation only an hour. In experiments on cats he removed the adrenals in two stages, and found that they then lived two to five hours after the operation. In each of three cases there was a fall in the blood sugar.

71. Eppinger, Falta and Rudinger: *Ztschr. f. klin. Med.*, 1909, **66**, 1.

72. Zuelzer: *Berl. klin. Wchnschr.*, 1909, **46**, 1209.

73. Von Fürth and Schwarz: *Biochem. Ztschr.*, 1911, **31**, 113.

74. Frugoni: *Berl. klin. Wchnschr.*, 1908, **45**, 1606.

75. Biedl and Offer: *Wien. klin. Wchnschr.*, 1907, **20**, 1530.

76. Frouin: *Compt. rend. Soc. de biol.*, 1908, **60**, 216.

77. Mayer: *Compt. rend. Soc. de biol.*, 1908, **60**, 219.

I have studied the effect of adrenal extirpation in the following way: The right adrenal, being the more difficult of the two to remove, is extirpated at the first operation. The animal is then allowed a week or more in which to make a complete recovery from the operation. Such dogs show no signs of adrenal insufficiency. At the second operation the pancreas is removed. When the blood sugar has risen to a diabetic level the left adrenal is extirpated. This is an easy operation, and can be done through a lumbar incision in twenty minutes. During the time that the animal survives, frequent blood sugar determinations are made, and the results plotted in a curve. Such an experiment on a dog is represented in Chart 1. It will be seen that following removal of the pancreas the blood sugar rose rapidly from the normal level (0.100 per cent.) to 0.305 per cent. Then the second adrenal (left) was removed. After a short rise, doubtless due to nerve stimulation during the operation, the blood sugar rapidly and uninterruptedly fell to normal. The dog survived the operation twenty-three hours, and during the first five or six hours after recovery from the anesthetic remained in good condition. This experiment has been repeated several times with the same result. The complete results of these experiments will shortly be published.

We see, therefore, that a considerable mass of evidence has accumulated to support the theory of a specific interaction between the adrenals and pancreas. None of it, however, is completely convincing. We shall revert to this question later on, after having considered the possibility that the adrenal secretion affects the power of the tissues, particularly of the muscles, to utilize sugar.

But first the explanation offered by Edmunds<sup>78</sup> and recently supported by the work of Mann and Drips<sup>79</sup> must be mentioned. Benedicente<sup>80</sup> and Pemberton and Sweet<sup>81</sup> believed that because there is a diminished flow of the external secretion of the pancreas following epinephrin injections, there was also an inhibition of the secretion of the islands of Langerhans. Edmunds, however, offered evidence in support of the view that the diminished pancreatic secretion is merely consequent on the vasoconstriction produced by epinephrin. The increased flow after adrenalectomy is due according to Mann and Drips merely to the changes in blood pressure, decreased temperature, etc., incident to the moribund condition. It is, therefore, clear that before the specific relationship between islands of Langerhans and adrenal medulla be accepted, the effect of alterations in the blood supply of the pancreas after adrenalectomy or epinephrin injections must be excluded.

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78. Edmunds: *Jour. Pharm. and Exper. Therap.*, 1911, **2**, 559.

79. Mann and Drips: *THE ARCHIVES INT. MED.*, 1915, **16**, 681.

80. Benedicente: *Arch. d. biol.*, 1905, **95**, 1.

81. Pemberton and Sweet: *THE ARCHIVES INT. MED.*, 1908, **1**, 628.



If it could be shown that the suprarenal secretion possesses the power to inhibit the oxidation of sugar by the tissues, it would strongly support the view, which has here and there cropped out in the literature of the subject, that in diabetes the basic fault is an excess of secretion by the adrenals or a diminished inhibition of this secretion by the pancreas. Experiments on the respiratory quotient and heat production supply the most significant facts in determining whether or not epinephrin affects glycolysis.

The respiratory quotient represents the ratio of the amount of carbon dioxid formed to the amount of oxygen required in the combustion of foodstuffs. Glucose, having sufficient oxygen to combine with all the hydrogen present to form water, will require for every molecule of carbon dioxid formed one molecule of oxygen. Plainly, then, the glucose respiratory quotient (R. Q.) is 1. In the case of protein the R. Q. is 0.81. Fat contains relatively less oxygen and consequently has a lower R. Q. This has been found to be 0.70. When, therefore, an animal is burning carbohydrate the R. Q. will be high; if he is burning only protein or protein and fat it will fall somewhere between 0.70 and 0.81.

Wilenko<sup>82</sup> studied the influence of sugar ingestion and epinephrin administration in rabbits under urethane anesthesia. He found (1) that epinephrin has little or no effect on the R. Q. of fasting rabbits, the carbohydrate stores having presumably been exhausted; (2) that epinephrin depresses the physiologic elevation of the R. Q. after administration of carbohydrate; (3) that intravenously administered glucose in epinephrin animals appears quantitatively in the urine. His conclusion is that epinephrin diminishes the ability of the organism to burn sugar. La Franka<sup>83</sup> had previously reported experiments in which he found a lowering of the R. Q. in phlorizin and pancreas glycosuria, but no change after epinephrin administration. Hári<sup>84</sup> starved dogs twenty-four to thirty-six hours, and then after curarizing them, injected epinephrin intraperitoneally and intravenously. He found that the epinephrin increased the R. Q., indicating that more sugar was burned after the injections than before. Fuchs and Roth,<sup>85</sup> in two cases of Addison's disease, found a distinct rise in the R. Q. after subcutaneous epinephrin injections. Similar results have been reported by Falta.<sup>85</sup> Lusk<sup>86</sup> has attempted to settle this question by experiments with the respiration calorimeter. He found that epinephrin administered twenty-one hours after food ingestion does not prevent the oxidation of glucose in a well-nourished dog. He also injected epi-

82. Wilenko: *Biochem. Ztschr.*, 1912, **42**, 44.

83. La Franka: *Ztschr. f. exper. Path. u. Therap.*, 1909, **6**, 1.

84. Hári: *Biochem. Ztschr.*, 1911, **38**, 23.

85. Falta: *Die Erkrankungen der Blutdrüsen*, 1913, p. 428.

86. Lusk: *THE ARCHIVES INT. MED.*, 1914, **13**, 673.



nephren during a period of glucose absorption, and found no depression of the R. Q. following the injections. During two experimental periods of six hours and five hours, respectively, he found the R. Q. to be 0.98 and 0.99.

The preponderance of evidence, therefore, so far as alteration in the respiratory quotient is concerned, favors the view that epinephrin does not inhibit the combustion of sugar. Efforts<sup>87</sup> to show that epinephrin can act as an antiferment, inhibiting the action of the glycolytic ferment of the blood, have been unsuccessful.

The observations cited above that the hyperglycemia in experimental pancreas diabetes rapidly disappears after extirpation of the adrenals, or is prevented by simultaneous adrenalectomy, have led to the assumption that in the absence of both pancreas and adrenals the animal is able to oxidize glucose. This is almost equivalent to saying that when the blood sugar of such an animal has fallen to a normal level his diabetes has been cured, and that death comes from adrenal insufficiency, even though the power of carbohydrate combustion has been restored. Such an assumption is gratuitous, because the mechanism regulating the mobilization of sugar is susceptible to so many influences — nervous, circulatory and chemical — that results after extirpation experiments are not convincing. In depancreatized dogs I have observed a return of the blood sugar to normal levels when an Eck fistula was created, and the stalk of the liver ligated. Chart 2 represents the blood sugar curve in such an experiment. The drop in the blood sugar on exclusion of the liver from the circulation is exactly similar to that produced by adrenal extirpation. Further evidence against the antiglycolytic action of epinephrin and the view that adrenalectomy restores to the animal with pancreas diabetes the power to burn sugar, is supplied by the experiment represented in Chart 3. In this experiment the right adrenal was extirpated on December 29. This produced no change in the blood sugar. One month later, after the animal had made a complete recovery, and seemed to be quite well, the pancreas was removed. Three days later the blood sugar was 260 mg. per 100 c.c. The left adrenal was then removed, and 2 gm. of glucose per kilo were injected intravenously. The blood sugar immediately shot up to 560 mg. per 100 c.c. If this animal had had his power of sugar consumption restored he should have shown a steady fall in the blood sugar curve, but this did not occur. The blood sugar took a sharp drop to a level somewhat above the level before the sugar injection and remained there until he died. The sugar excreted in the urine during the last twenty-four hours was 101.8 gm., while during the preceding twenty-four hours it had been 64.9 gm. Since 33.6 gm.

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87. Mackenzie: *Jour. Exper. Med.*, 1915, **22**, 757.

were injected it is evident that the intravenously administered glucose was recovered quantitatively in the urine, and there is no evidence that any sugar was burned. Moreover, sections of liver and striated muscle taken immediately after death, fixed in absolute alcohol and stained by Best's picrocarmin method, showed no evidence of glycogen. One may conclude from this that not only was there no restoration of glycolytic power, but also no return of the power to store glycogen after extirpation of the adrenals.

It is evident from this review of the experimental work designed to clear up the problems of the relation of the adrenal secretion to glycolysis, that the weight of evidence favors the view that they are unrelated. Wilenko's experiments on the respiratory quotient changes after epinephrin injections have not been confirmed by other investigators, and Zuelzer's conclusion, founded on a disappearance of reducing substance from the blood after adrenal extirpation, is based on insufficient evidence. We must conclude, therefore, that epinephrin does not interfere with the process of sugar oxidation by the tissues.

#### CONCLUSIONS

To conclude, it seems that we are justified, from the work that has been done, in accepting the following view of the relation of the suprarenal system to carbohydrate metabolism:

1. Nervous stimuli, especially of the sympathetic, represented by piqure or splanchnic stimulation, are followed by an increased secretion of epinephrin, and this hyperadrenalinemia like that following epinephrin injections, causes hyperglycemia and glycosuria in part by inhibiting glycogenesis, and in part by furthering glycogenolysis.

2. The hyperglycogenolysis thus produced is dependent partly on a direct stimulation of the liver cells and partly on its action in rendering the receptive material between the sympathetic nerve endings and the liver cells more sensitive to nervous stimulation.

3. Epinephrin does not produce its effects by inhibiting glycolysis, and the disturbances in sugar metabolism following its administration have little or nothing to do with the loss of glycolytic power, which is probably a part of the altered metabolism in diabetes mellitus.

4. A specific physiologic relation between the islands of Langerhans and the adrenal medulla is unproved.

5. Adrenalectomized dogs show a diminution of the power to form glycogen from glucose.

6. Following extirpation of the adrenals in depancreatized dogs there is a rapid disappearance of hyperglycemia.

7. Sugar administered to such animals is neither oxidized nor stored as glycogen, but appears quantitatively in the urine.

# A STUDY OF ETHYLHYDROCUPREIN (OPTOCHIN) IN THE TREATMENT OF ACUTE LOBAR PNEUMONIA \*

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## SYNOPSIS

Historical.

Scope of the problem and general considerations.

Character of the cases and their general treatment.

Technic employed; interpretation of results.

Dosage of optochin:

I. Oral administration entirely.

The various methods of dosage used; comparison.

Relationship between body weight of patient and appearance of  
bactericidal action in the serum.

II. Combined intramuscular and oral administration.

Absorption and elimination.

Fastness.

Toxic effects.

Influence on the clinical course of the disease:

1. Occurrence of "spreads."

2. Pneumococcal septicemia.

3. Duration of the disease.

4. Mortality rate.

Discussion.

Conclusions.

## HISTORICAL

The valuable studies of Morgenroth and his co-workers<sup>1</sup> on the chemotherapy of the quinin alkaloids have shown that ethylhydrocuprein (optochin) has a specific bactericidal effect on the pneumococcus in vitro, and that this compound exerts a protective and curative action in animals experimentally infected with virulent strains of that micro-organism. The specific germicidal action of ethylhydrocuprein on the pneumococcus is greater than that of any other compound of the series examined, namely, quinin, hydroquinin (methylhydrocuprein),

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\* From the Hospital of The Rockefeller Institute for Medical Research, New York.

\* Paper read in abstract before the Society for Serology and Haematology, New York, April 7, 1916.

1. Morgenroth, J. and Levy, R.: Berl. klin. Wchnschr., 1911, **48**, 1560 and 1979. Morgenroth, J., and Kaufmann, N. M.: Centralbl. f. Bakteriöl., 1912, **54**, Ref., Part 69; Ztschr. f. Immunitätsf., 1913, Orig., **18**, 145. Morgenroth, J., and Bumke, E.: Deutsch. med. Wchnschr., 1914, **40**, 538. Engewer, T.: Ztschr. f. Hyg. u. Infektionskr., 1913, **73**, 194. Gutmann, L.: Ztschr. f. Immunitätsf., 1912, Orig., **15**, 625. Levy, R.: Berl. klin. Wchnschr., 1912, **49**, 2486.



isopropylhydrocuprein, isobutylhydrocuprein and isoamylhydrocuprein. Indeed, if the value of ethylhydrocuprein in this respect be represented by 1, then that of isopropylhydrocuprein =  $\frac{1}{2}$  to  $\frac{1}{4}$ , isoamylhydrocuprein =  $\frac{1}{20}$  and quinin =  $\frac{1}{150}$ . Isbutylhydrocuprein is no more potent in this respect than quinin itself, nor does hydroquinin appear to be of much greater value.

Shortly after the publication of these studies of Morgenroth and his collaborators, the drug ethylhydrocuprein was placed on the market under the trade name of "optochin" (the free base, the hydrochlorid, and, later, the salicylicacidester) and use was made of it in the treatment of acute lobar pneumonia in man. In the great majority of recorded cases it was administered by mouth.

Little being known regarding the dosage of this drug in man, it is not surprising that in several of the earlier cases its exhibition led to alarming and untoward symptoms, such as tinnitus, deafness, amblyopia or amaurosis, and that, even at the present time, opinion among clinicians is by no means unanimous regarding either its value or its dosage. The results of studies made by one of us (M.) on the action of ethylhydrocuprein on type strains of the four main serologic groups of pneumococci,<sup>2</sup> and on the pneumococcal action acquired by the serum of rabbits after its administration,<sup>3</sup> led to the present investigation. Its main object was to discover how the dosage of the drug might be regulated in man. In this endeavor we were helped by a study of the literature, for the clinical experience therein recorded seemed to fix the upper limit of dosage, beyond which it was not safe to go, at about 1.5 gm. per twenty-four hours, given by mouth. In no case reported in the literature have we been able to discover the weights of the patients treated, and, accordingly, we have been forced to take this upper limit as that for a patient of average size.

It is difficult to arrive at a valuation of the drug in acute lobar pneumonia from a perusal of the published treated cases, because of contradictory opinions; whereas Wright<sup>4</sup> thinks it inefficacious or doubtfully efficacious, and Parkinson<sup>5</sup> states that it has little or no effect on the course of pneumonia in man, many authors express favorable opinions. It would appear that the earlier writers on this subject used unsuitable doses, while the later observers used a dosage more nearly approaching the optimum, and perhaps for this reason obtained better results.

2. Moore, H. F.: Jour. Exper. Med., 1915, **22**, 269.

3. Moore, H. F.: Jour. Exper. Med., 1915, **22**, 551.

4. Wright, A. E., Morgan, W. P., Colebrook, L., and Dodgson, R. W.: Lancet, London, 1912, **2**, 1633 and 1701.

5. Parkinson, J.: Ztschr. f. Chemotherapie u. verw. Geb., 1913, Orig., **2**, 1.

We have collected from the literature 787 cases,<sup>6</sup> the details of which are more or less completely available. They are reproduced in tabular form in Table 1. Empyema in patients treated with the drug does not seem to have been a frequent complication, 1.9 per cent. The total mortality in the 787 cases recorded is 12.96 per cent., a figure in itself somewhat encouraging in the use of the drug.

#### SCOPE OF THE PROBLEM AND GENERAL CONSIDERATIONS

The present investigations had as its main object the solution of the following problems:

1. Is it possible to confer on the serum of patients of average size, by the administration of 1.5 gm. of ethylhydrocuprein per twenty-four hours, the power of killing pneumococci in the test tube?

2. If so, what is the optimum method of administration of the drug, both as regards the route, and the regulation of the size and spacing of the individual doses, as determined by a study of this acquired bactericidal property of the serum?

3. What is the effect of the drug on the course and mortality of acute lobar pneumonia in man, when the influence of the particular immunologic group of the infecting pneumococcus on the natural course of the disease is taken into consideration?

It is logical to suppose that if ethylhydrocuprein has any special value in the treatment of acute lobar pneumonia its efficacy must be due to its specific pneumococcidal action. Unless it can be given in such amounts that there is produced within the human organism a condition whereby pneumococci are destroyed in situ, or, at least, prevented from local or general migration, and from the establishment of their growth in previously uninfected regions, it seems unlikely that marked therapeutic results can be expected from its use.

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6. Baermann, G.: *Ztschr. f. exper. Path. u. Therap.*, 1914, **15**, 476. Bieling: *Therapie d. Gegenw.*, 1915, **56**, New Series **17**, 203. Dünner, L., and Eisner, G.: *Therap. d. Gegenw.*, 1916, **57**, New Series **18**, 41. Von Dziembowski, S.: *Deutsch. med. Wchnschr.*, 1915, **41**, 1571. Frank, E.: *Berl. klin. Wchnschr.*, 1915, **52**, 421. Frank, G. V.: *Zentralbl. f. inn. Med.*, 1916, **37**, 265. Fränkel, A.: *Berl. klin. Wchnschr.*, 1912, **49**, 664; *Therap. d. Gegenw.*, 1915, **56**, New Series **17**, 1. Hess, O.: *München. med. Wchnschr.*, 1915, **62**, 1528. Kaufmann, M.: *München. med. Wchnschr.*, 1915, **62**, 291. Lapinski, J.: *Therap. Monatsh.*, 1915, **29**, 103. Lenné: *Berl. klin. Wchnschr.*, 1913, **50**, 1984. Leschke, E.: *Deutsch. med. Wchnschr.*, 1915, **41**, 1359. Loeb, A.: *Berl. klin. Wchnschr.*, 1915, **52**, 1108. Loewe, E., and Meyer, F.: *Berl. klin. Wchnschr.*, 1915, **52**, 1018. Manliu, J.: *Berl. klin. Wchnschr.*, 1916, **53**, 58. Mendel: *München. med. Wchnschr.*, 1915, **62**, 740. Moritz: *Ztschr. f. ärzt. Fortbild.*, 1915, **12**, 321. Oliver, G. H.: *Brit. Med. Jour.*, 1916, **1**, 580. Peiper, A.: *Berl. klin. Wchnschr.*, 1915, **52**, 396. Rosenow, G.: *Berl. klin. Wchnschr.*, 1915, **52**, 393. Rosenow, G.: *Deutsch. med. Wchnschr.*, 1915, **41**, 791. Rosenthal, F.: *Therap. d. Gegenw.*, 1915, **56**, New Series, **17**, 181. Schottmüller: *Berl. klin. Wchnschr.*, 1915, **52**, 758. Silbergleit, H.: *Berl. klin. Wchnschr.*, 1915, **52**, 1231. Simon, A.: *Deutsch. med. Wchnschr.*, 1915, **41**, 643. Vetlesen: *Berl. klin. Wchnschr.*, 1913, **50**, 1473.

TABLE 1.—SUMMARY OF REPORTED CASES OF ACUTE LOBAR PNEUMONIA TREATED WITH OPTOCHIN<sup>a</sup>

Author	Num-ber of Cases	Summary of Dosage per 24 Hours, in Gm.*	Disturbances of Vision	Disturbances of Hearing	Vomit-ing	Empy-ema	Mortal-ity	Remarks
Baermann, G. ...	34	3 cases: 1 × 0.75; hydrochlorid intravenously	.....	.....	.....	.....	1	
		5 cases: 2.4 × 0.5, base (in oil) intramuscularly	.....	.....	.....	.....	2	
		11 cases: 2.3 × 0.5 or 4 × 0.25 base, (in oil) intramuscularly and 20-40 c.c. convalescent serum subcutaneously	.....	.....	.....	.....	2	One patient had miliary tuberculosis
		7 cases: 2.4 × 0.5; 6.8 × 0.25; hydrochlorid by mouth	.....	.....	.....	1	1	
Bieling.....	16	8 cases: 2.4 × 0.5; 6 × 0.25; hydrochlorid by mouth and 20-50 c.c. serum subcutaneously	.....	.....	.....	.....	2	
		3.4 × 0.5 hydrochlorid	.....	.....	.....	.....	12	Smaller doses in children
Dunnet, L. and Eisner, G.	100	3 × 0.5; 6 × 0.25; hydrochlorid; when any toxic symptoms appeared the dose was diminished to 6.8 × 0.125-0.15, or drug was discontinued	(5 "glimmering" before eyes); 3 amblyopia; 1 amaurosis; all transient	9 tinnitus.....	4	2		One lung abscess
Frank, E. ....	3	4 × 0.5.....	1 amaurosis (improvement before death)	.....	.....	.....	1	
Frank, G. V. ....	40	3 × 0.5; 6 × 0.25; 4.5 × 0.25, base	1 amaurosis; 1 severe amblyopia (improvement before death); 3 slight amblyopia	1 deafness.....	.....	.....	10	
Fränkel, A. (I)....	21	2.5 × 0.5, hydrochlorid	3 amblyopia; optochin discontinued; transient	.....	1 or 2	.....	4 (severe cases)	
Fränkel, A. (II)...	13	3 × 0.5; hydrochlorid	.....	1 tinnitus.....	.....	.....	1	



Hess, O. ....	81	3 × 0.5; 6-8 × 0.2; 6 × 0.25; hydrochlorid	6 amblyopia, transient; 1 amaurosis; optochin stopped; recovery; 1 amaurosis; almost complete recovery	Occasional difficulty of hearing and tinnitus	.....	.....	10†	4-5 × 0.2 gm. for 1-2 days after temperature became normal
Kaufmann, M. ....	5	4 × 0.5; 6 × 0.25; hydrochlorid	1 amaurosis; transient.....					
	14	5 × 0.3; hydrochlorid	.....	Difficulty of hearing and tinnitus in several cases	Several cases	.....	2†	Continues optochin 1-2 days after temp. becomes normal; continuous day and night dosage to prevent "fastness."
Lapinski, J. ....	35	3 × 0.5; 5-6 × 0.5; 8 × 0.5; 4 × 0.5; 12 × 0.5 (one case); hydrochlorid, base and salicylic acid ester	1 slight amblyopia; 1 amaurosis; 1 amaurosis; all transient	.....	Occasional	1	5	
Lenné.....	17	1-4 × 0.4; 2-4 × 0.5; hydrochlorid	1 anaurosis, transient; (dilatation of pupils in several cases)	1 difficulty in hearing, transient	.....	1	2	Smaller doses to children under 15 years
	18	2-3 × 0.4-0.5, hydrochlorid, + serum (20-40 c.c.), 2 intravenous injections of 0.2 gm. each in one patient	.....	.....	1	.....	3	
Leschke, A. ....	5	10 × 0.2; optochin salicylic acid ester; when temp. drops 5 × 0.2	1 amblyopia, transient; (several slight disturbances; transient)	Several times tinnitus				
Loeb, A. ....	24	6 × 0.25; hydrochlorid and salicylic acid ester	.....	1 tinnitus and difficulty of hearing, transient	1	1	2	
Loewe F. and Meyer, F.	43	4-6 × 0.25; hydrochlorid	2 slight amblyopia; transient. (widen- ing of pupils; optochin was discontinued)	1 tinnitus.....	.....	.....	2‡	Milk diet with base
Manlieu, J. ....	12	6 × 0.25; hydrochlorid or base	1 amblyopia; transient.....	Occasional tinnitus	.....	.....	.....	Milk diet
Mendel.....	12	5 × 0.3, base	Amaurosis; vision improved, but later declined; vision still much impaired 1 year after the drug was given	Tinnitus and partial deafness during administration				
Moritz.....	28	8 × 0.5						
Oliver, G. H. ....	1	8 × 0.3 (total 120 grains; 7.7 gm.)						

\* By mouth unless otherwise stated.

† Two mixed infection with streptococci.

‡ One mixed infection with streptococci; one Grave's disease.

§ One nephritis; one pericarditis.

TABLE 1.—SUMMARY OF REPORTED CASES OF ACUTE LOBAR PNEUMONIA TREATED WITH OPTOCHIN—(Continued)

Author	Number of Cases	Summary of Dosage per 24 Hours, in Gm.*	Disturbances of Vision	Disturbances of Hearing	Vomiting	Emphysema	Mortality	Remarks
Parkinson, J. ....	9	1.4 × 0.5; hydrochlorid by mouth; 1 × 0.125—3 × 0.5; hydrochlorid subcutaneously	(3 dilatation of pupils).....	.....	.....	2	2	
Peiper, A. ....	31	2 × 0.25; 2.3 × 0.5; 6 × 0.25	1 slight amblyopia, transient; 1 amaurosis, transient	.....	.....	.....	6	
Rosenow, G. (I)...	26	6 × 0.25; hydrochlorid and salicylic acid ester	.....	.....	.....	1	2	
Rosenow, G. (II)...	34	6 × 0.25 hydrochlorid by mouth; 8 × 0.2 hydrochlorid by mouth; 6-8 × 0.25 salicylic acid ester by mouth; 6-8 × 0.25 base, by mouth; 1 × 0.5 hydrochlorid intraven.	.....	1 tinnitus, 1 difficulty in hearing	.....	4	2	Continues drug for a few days after temperature drops
Rosenthal, F. ....	6	3 × 0.5; 6 × 0.25; hydrochlorid	.....	.....	.....	.....	1 <sup>†</sup>	
Silbergelt, H. ....	49	6 × 0.25; 6 × 0.2; hydrochlorid	1 slight amblyopia.....	Tinnitus.....	.....	1	5	
Simon.....	57	3.6 × 0.25; ester and hydrochlorid (exceptionally 3 × 0.5)	2 amblyopia, transient.....	12 difficulty in hearing, transient	.....	.....	15	Better results in early cases
Schottmüller.....	3	1.5 gm. per 24 hours	.....	.....	.....	.....	1	
Vetlesen.....	9	3 × 0.5 hydrochlorid	.....	3 tinnitus and deafness, transient	.....	.....	.....	
Warburg.....	41	3 × 0.5; 6 × 0.25; hydrochlorid base, salicylic acid ester	.....	Tinnitus and difficulty in hearing in a few cases	.....	1	6	
Totals.....	787	.....	38 (4.57 per cent.) amaurosis or amblyopia	.....	.....	15 (1.9%)	102 (12.96%)	

<sup>†</sup> Typhoid, convalescent.

Wright<sup>4</sup> was the first to show that the serum of human patients receiving the drug by mouth destroyed pneumococci in the test tube. It has been shown by one of us (M.)<sup>8</sup> that the serum of normal rabbits which had received a single comparatively large but well tolerated dose of ethylhydrocuprein hydrochlorid acquires an inhibitory and bactericidal action on pneumococci in the test tube; furthermore, that this bactericidal action resulting from a single dose lasts but a couple of hours in the living animal, and then disappears. When rabbit serum possessing this property is inoculated with a small amount of an eighteen-hour broth culture of pneumococcus and incubated at 37 C., the destruction of any considerable number of pneumococci requires a period of several, often as many as forty, hours, the time required depending in part on the degree of bactericidal power which the serum possesses and in part on the number of pneumococci present. It follows, therefore, that, in order to destroy pneumococci within the living animal, the drug should be repeatedly administered in suitable doses, at comparatively short intervals of time, in order that the pneumococidal action in the circulating fluids may be continuously preserved for the necessary length of time.

Any effort to cure a pneumococcal septicemia or pneumonia through the administration of ethylhydrocuprein must aim at (1) the production of a pneumococidal action in the blood serum; (2) its maintenance as constantly as possible over a period of time sufficiently long to insure the destruction of all the infecting pneumococci; and, (3), the production of a concentration of the drug within the body which is harmless to any part thereof.

Marked success has not attended our efforts to cure pneumococcal septicemia in the rabbit, owing to the susceptibility of these animals to such amounts of the drug as are necessary for the production of a constant bactericidal action in the blood stream for a sufficiently long period.

In acute lobar pneumonia it should not be surprising if the time required for such an agent as optochin to destroy all the pneumococci in the lung lesion were greater than that necessary to destroy those circulating in the blood stream, because it is possible that the majority of pneumococci in the lung lesion may be mechanically protected by the exudate from the action of the drug. Indeed, this would seem to be, in part, the explanation of the fact, pointed out by Morgenroth,<sup>7</sup> Leschke<sup>8</sup> and others, that the results of optochin treatment in lobar pneumonia are better the earlier after the onset of the disease it is instituted; in other words, before any considerable consolidation of

7. Morgenroth, J.: Berl. klin. Wchnschr., 1914, **51**, 1829 and 1865.

8. Leschke, E.: Deutsch. med. Wchnschr., 1915, **41**, 1359.



the affected lung has taken place. On the other hand, we might expect that a much shorter time would be required for the destruction of pneumococci circulating in the blood stream.

There is still another consideration of importance in the treatment of lobar pneumonia with optochin, namely, the possibility that a dosage which is too small, apart from failing to confer a pneumococcidal action on the circulating blood, may give rise to conditions which favor the acquisition by the infecting pneumococci of a condition of resistance or "fastness" to the drug, so that even a later increase in dosage may be insufficient to destroy the bacteria and hence be of no avail.

Morgenroth and Kaufmann<sup>9</sup> have shown that, in mice infected with pneumococci and insufficiently treated with ethylhydrocuprein, the pneumococci are able to react against the drug so that, after four passages through such insufficiently treated mice, the strain may become completely resistant, or "fast," to its action. A similar phenomenon can be observed in the test tube. Tugendreich and Russo<sup>10</sup> have shown that, by subjecting pneumococci for two hours daily, at room temperature, to gradually increasing and at first sublethal concentrations of optochin and incubating in serum-broth in the intervals, the micro-organisms could be educated, so to speak, in ten or twelve days, to grow in comparatively high concentrations of the drug. We, ourselves, carrying out similar observations at 37 C., have seen, in six subcultures made in gradually increasing concentrations of optochin, a strain of pneumococcus become capable of growing in a concentration of the drug greater than that previously sufficient to kill it.

We feel that these phenomena are of significance in the present problem, namely, the chemotherapy of pneumonia with ethylhydrocuprein, since it is possible that here the conditions may at times favor the micro-organism, in that the pneumonic exudate may conceivably afford some degree of protection to the pneumococci from the circulating chemotherapeutic agent. Assuming that this may be so, it is possible, in the treatment of pneumonia with optochin, that the pneumococci may be hindered from reacting against and becoming fast to the drug, by giving to the patient such doses of optochin as will, within limits of safety, confer on the circulating blood the maximum bactericidal action in the shortest space of time, and by maintaining this action as nearly as possible at a constant level throughout the treatment. In other words, the development on the part of the parasite of a condition of fastness is to be regarded as a vital action, and one object of the therapy is to subject the infecting pneumococci to such conditions as

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9. Morgenroth, J., and Kaufmann, M.: *Ztschr. f. Immunitätsf.*, 1912, Orig., **15**, 610.

10. Tugendreich, J., and Russo, C.: *Ztschr. f. Immunitätsf.*, 1913, Orig., **19**, 156.

will prevent them from exercising any vital activity. Fortunately, such an adaptation of the parasite to these influences seems to require a considerable space of time for its development; apparently, several days of exposure to nonfatal concentrations of optochin are necessary before the pneumococcus acquires such a degree of "fastness" that it can carry on its activities in the presence of the degree of bactericidal action that may readily be produced in the circulating fluids by the administration of optochin. In the actual treatment of disease, therefore, with such a chemotherapeutic agent as optochin, it would seem desirable to avoid such a low concentration of the drug in the blood as would permit the pneumococci to develop the property of "fastness." A case bearing on this point will be cited later.

Bearing in mind the foregoing considerations, one is led to believe that the ideal to be attained in the use of ethylhydrocuprein in acute lobar pneumonia is to regulate the administration of the drug so that,

(1) within the limit of safety, a maximum bactericidal action may appear in the circulating blood within the shortest possible time after the commencement of the specific treatment; and,

(2) the bactericidal action may be maintained as constantly as possible, as long as the administration of the drug is required.

The first object, namely, the rapid production of the bactericidal action in the blood stream, might be attained by an intravenous injection. This, however, did not commend itself to us, because the work of one of us (M.),<sup>8</sup> on intravenous injection of the drug in rabbits, has shown that toxic signs may result from doses so small that but little bactericidal action results in the serum. Boecker,<sup>11</sup> also, after intravenous administration, was unable to demonstrate considerable bactericidal action in the serum of rabbits, and was only slightly more successful with guinea-pigs. Further, a case was reported by Smith and Fantus<sup>12</sup> in which intravenous injections of optochin resulted in numbness of the lower extremities, impaired vision and deafness, and in which death was attributed by these authors to cardiac depression, which was considered to be in part due to the intravenous administration of the drug. Baermann,<sup>13</sup> however, has given intravenous injections of single doses of 0.75 gm. of the drug without recording ill effects. Rosenow<sup>14</sup> also used intravenous injection of 0.5 gm. Likewise, subcutaneous injection did not seem to us desirable, in general, in view of the painful infiltration to which it may give rise.<sup>8</sup> As will be mentioned below, intramuscular administration has also been tried in the present study, with unsatisfactory results. Consequently, oral

11. Boecker, E.: *Ztschr. f. Immunitätsf.*, 1915, Orig., **24**, 148.

12. Smith, M. I., and Fantus, B.: *Jour. Pharmacol. and Exper. Therap.*, 1916, **8**, 53.

13. Baermann, G.: *Ztschr. f. exper. Path. u. Therap.*, 1914, **15**, 476.

14. Rosenow, G.: *Deutsch. med. Wchnschr.*, 1915, **41**, 791.



administration has been adopted as a general method; the absorption of the hydrochlorid of the drug from the alimentary canal seems to be so rapid that the requirement under discussion, namely, a rapid appearance of the bactericidal action in the serum, is capable of fulfilment by using this route.

The proposed ideal of constant maintenance of the bactericidal action required study of the regulation of the size and spacing of the individual doses designed to produce this result.

If 1.5 gm. for an individual of average size be decided on as the total amount of the drug to be given per twenty-four hours, this quantity may be subdivided in several ways. The method of administration most likely to give rise to a fulfilment of the condition outlined above is that of giving one or several initial relatively large doses of the drug (for example, 0.45 to 0.5 gm.), followed by several smaller ones at regular intervals. It is less likely that several small doses of, say 0.15 gm., would produce a bactericidal action within as short a space of time as the former method. But, whatever method of regulating the doses be employed, if the rates of absorption and elimination or destruction of the drug be comparable in different individuals, then the body weight, through its influence on blood volume, should play an important part in the resulting concentration of the drug in the blood stream; in other words, the concentration of optochin should be greater in those patients who receive a greater amount per kilogram of body weight. If these views are correct, a certain minimum amount of optochin must be absorbed per kilo of body weight within a definite time in order that any bactericidal action in the blood stream may result. Evidence in favor of such a view will be brought forward later. It will suffice now to suggest that, in order to produce in the blood stream the desired bactericidal action, the possibility of a relationship between the body weight of the patient and the amount of optochin given per twenty-four hours must be taken into consideration.

#### CHARACTER OF THE CASES AND THEIR GENERAL TREATMENT

The present study comprises observations on thirty-two patients suffering from acute lobar pneumonia, admitted to the wards of the Hospital of The Rockefeller Institute during the months November, 1915, to July, 1916. Each of these thirty-two patients received optochin<sup>15</sup> hydrochlorid, and in twenty-eight of them a detailed study

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15. The optochin used in this study was obtained through Merck & Co., New York, was manufactured by Vereinigte Chininfabriken, Zimmer & Co., Frankfurt a. M., and the bottles containing it bore the number 11,695. It was a white powder. We have tested experimentally other specimens of optochin which were more or less yellowish-white in color, but they apparently were not as potent as the drug used in the present study and one of them was found to contain impurities. It is important that the drug should be free from impurities if a uniform system of dosage is to be employed.



of the blood serum was made with reference to the presence or absence of bactericidal power for the pneumococcus. With one exception, a strain of pneumococcus was isolated from every case, and differentiated according to the biologic classification proposed by Dochez and Gillespie<sup>16</sup> and later Avery.<sup>17</sup> The cases treated were due to infection with representatives of all the main groups of pneumococci, although the majority were infected with strains belonging to Group II and Group III (*Pneumococcus mucosus*). Only two cases of infection with pneumococci belonging to Group I were treated with optochin, and of these, one received, in addition, several doses of antipneumococcus serum (Group I) intravenously; all other cases of infection with strains of this group were purposely not treated, owing to the employment of other therapeutic methods. Five cases of infection with strains of Group II were treated, in addition to the optochin, with "extract" of the type homologous antipneumococcus serum,<sup>18</sup> which was prepared according to Chickering's<sup>19</sup> modification of the method described by Gay<sup>20</sup> and the former investigator. Two cases due to infection with *Pneumococcus mucosus* (Group III) were likewise treated with the extract of the corresponding antiserum<sup>21</sup> as well as with optochin. No attempt was made to select the patients for treatment, so that, for purposes of comparison, this series may be considered to be a fairly representative one. The extremes of age were 15 and 66 years, the average age 37.9 years. The incidence of the cases according to decade is given in the following tabulation:

Decade	No. of Cases
11 to 20 inclusive.....	5
21 to 30 inclusive.....	7
31 to 40 inclusive.....	7
41 to 50 inclusive.....	5
51 to 60 inclusive.....	5
61 to 70 inclusive.....	3

Apart from the optochin or serum therapy, the general treatment of the patients was that usually carried out in this hospital. Digipuratum was used when indicated, and it may be stated that this drug has no effect on the bactericidal action in the serum resulting from optochin therapy, nor does it confer such a property itself. The use of camphor was avoided except in two cases towards the end of the

16. Dochez, A. R., and Gillespie, L. J.: Jour. Am. Med. Assn., 1913, **61**, 727.

17. Avery, O. T.: Jour. Exper. Med., 1915, **22**, 804.

18. Such extract has no bactericidal action on the pneumococcus in the test tube.

19. Chickering, H. T.: Jour. Exper. Med., 1915, **22**, 248.

20. Gay, F. P., and Chickering, H. T.: Jour. Exper. Med., 1915, **21**, 389.

21. The particular serum from which this extract was obtained was kindly supplied by Dr. Wadsworth of the New York State Board of Health.

treatment because Rosenthal<sup>22</sup> has shown that its administration diminishes the curative effect of optochin in animals infected with pneumococci. It does not appear that any particular diet has an important effect on the bactericidal action resulting in the serum of patients treated with optochin hydrochlorid.

In the present study the free base of ethylhydrocuprein or the salicylicacidester has not been used; the hydrochlorid alone has been employed.

#### TECHNIC EMPLOYED; INTERPRETATION OF RESULTS

The bactericidal action of the serum was studied as follows: Specimens of blood were obtained from the veins at the elbow, before and at frequent intervals during the administration of the drug. The blood was allowed to clot and to stand in the refrigerator over night; the serum was then separated and kept in the refrigerator until all specimens had been assembled, when the tests were carried out.<sup>23</sup> In two cases in which the patients had positive blood cultures, the serum was heated at 56 C. for thirty minutes in order to kill any living pneumococci that might be present; except for these instances the serum was not inactivated, since the bactericidal power of the serum of patients who have received optochin is not appreciably increased or diminished by the process of inactivation. Serum was used for the tests after it had been ascertained that the whole blood was no more bactericidal than the former. Where one specimen of blood was obtained in the interval between two doses of optochin, as a general rule the bleeding immediately preceded the administration of the second dose.

Virulent laboratory strains of pneumococci, long accustomed to growth outside the body, were used for the tests. Such strains are more resistant to optochin than strains freshly isolated from the human body. In the majority of instances the particular strain used belonged to an immunologic group other than that causing the infection. In cases in which the infecting micro-organism belonged to the same group as that of the strain used for the bactericidal tests, agglutination tests (microscopic and macroscopic) were carried out with the patient's serum, in order to rule out the possibility that the patient had acquired agglutinins within his serum, the presence of which might give rise to a simulation of bactericidal action, thus introducing an obvious fallacy.

Bactericidal substances for the pneumococcus are not found among the antibodies demonstrable about the time of the crisis in patients with acute lobar pneumonia, nor is an artificially prepared immune serum itself pneumococcal; hence, any bactericidal action observed in the serum must have been due to the optochin treatment.

Most of the tests were carried out with cultures from four to six hours old. The age of the culture selected for carrying out bactericidal tests is by no means an unimportant detail. In a previous communication one of us (C.)<sup>24</sup> has reported the results of an investigation of the nature of bacterial lag, as it occurs in bouillon cultures of pneumo-

22. Rosenthal: Berl. klin. Wchnschr., 1915, **52**, 709.

23. The serum retains, undiminished, the bactericidal action resulting from the administration of optochin for a considerable period of time when kept in the refrigerator.

24. Chesney, A. M.: Jour. Exper. Med., 1916, **24**, 387.



coccus; in that work it was shown that actively growing (four to eight hours at 37 C.) cultures of pneumococcus, on transplantation into new mediums, exhibit no delay in growth but continue to grow rapidly; whereas, cultures twelve hours and older, on transplantation, require several hours (one to four or more) before rapid growth begins to take place. Furthermore, evidence was therein presented which indicates that this initial inhibition of growth, or "lag," exhibited by older cultures of pneumococci, is due to an injury caused by the action of the metabolic products of the bacteria themselves on that structure or function of the bacteria which has to do with the growth of the micro-organisms. On the bases of the facts brought out in that investigation, it was suggested that, in determining the bactericidal power of any agent, it might be desirable to carry out the tests with "young" cultures; that is to say, with such cultures as had not sustained an injury to their powers of growth and would, therefore, on transplantation, exhibit no lag. Otherwise, if cultures, the individuals of which had already been partially injured, were subjected to the action of a bactericidal agent, one would have to deal with a summation of effects, which, in turn, might give rise to an exaggerated idea of the efficacy of the agent tested. Support for this view is offered by the work of Chick<sup>25</sup> who has presented evidence which indicates that the individual organisms of a three-hour culture of *B. paratyphosus* are more resistant to the action of disinfectants than are those of a twenty-four-hour culture of the same strain. We shall present evidence later to show that the organisms of a four-hour culture of pneumococcus are more resistant to a bactericidal agent (optochin) than those of a twenty-hour culture of the same strain.

Moreover, the use of an actively growing culture affords the opportunity of measuring more accurately the degree of inhibitory or bactericidal power of the specimens tested, and thus, possibly, of estimating the relative amounts of the bactericidal agent present.

In actual practice, in the present study, it was found best to use a broth culture of pneumococcus which had been incubated at 37 C. for from four to six hours, so that it was just beginning to show a faint turbidity. Usually 0.001 c.c. of this culture was inoculated into each of the tubes containing 3.0 c.c. of the various specimens of serum to be tested. After all the tubes had been inoculated, 0.5 c.c. was removed from each, mixed with 20 c.c. of melted 1 per cent. glucose agar which had previously been cooled to 40 C., and plated at once. This amount of agar was sufficient to dilute the bactericidal agent in the serum to such an extent as to allow the pneumococci to grow on the plates. The tubes were then incubated in the dark in the water bath at 37 C. and, at suitable intervals thereafter, an equal amount (0.5 c.c.) was again removed from each tube and plated. All plates were incubated forty-eight hours and then the number of colonies on each was counted. It should be noted that "young" (four- to eight-hour) cultures of pneumococci contain many short

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25. Chick, H.: Jour. of Hyg., 1908, 8, 92.



chain forms and that each chain would give rise to only one colony on the plate. Nevertheless, since the specimens of serum of each case are tested with the same culture, the results in any one case are comparable; and, where the cultures used in testing the serums of different patients are of the same age, and grow at approximately the same rate, the results are comparable in different cases.

#### INTERPRETATION OF RESULTS

When actively growing pneumococci are subjected for twenty-four hours at 37 C. to the action of the serum of patients who have received optochin, several phenomena may take place. The micro-organisms may show, (1) unrestricted growth; (2) an initial temporary inhibition followed subsequently by unrestricted growth; (3) complete inhibition with little or no destruction of bacteria; or, finally, (4) there may be actual bactericidal action. The three phenomena last mentioned shall be hereafter designated "temporary inhibition," "complete inhibition," and "bactericidal action," respectively. They represent variations in degree of the same general process, namely, toxic action of optochin on the pneumococcus. Before attributing significance to the variations in pneumococcidal or inhibitory power, it is essential to determine whether or not they represent differences in the amount of optochin present in the serum. In order to ascertain this point, bactericidal tests were conducted with known concentrations of optochin in normal horse serum and "young" (four hours at 37 C.) and "old" (twenty hours at 37 C.) bouillon cultures of pneumococcus. The details of one of these experiments follows.

*Experiment 1.*—Varying dilutions of optochin in normal horse serum were made, ranging from 1:50,000 to 1:5,000,000. Into separate tubes containing 6 c.c. each of these dilutions there was inoculated 0.002 c.c. of a four-hour (37 C.) broth culture of pneumococcus, Group II. After thorough mixing, 0.5 c.c. was removed from each tube separately and plated in 1 per cent. glucose agar. The tubes were then incubated in the water bath at 37 C. and at suitable intervals 0.5 c.c. was removed from each tube and plated separately. All plates were incubated for forty-eight hours before counting. The same procedure was carried out with the same culture of pneumococcus when it was twenty hours old (37 C.), in order to contrast the behavior of the two under the same conditions (inoculation 0.0001 c.c.). The growth of the two cultures in the varying dilutions of optochin in normal horse serum is shown in Table 2.

Table 2 shows that dilutions of optochin in normal horse serum, varying in strength from 1:5,000,000 to 1:2,000,000, inclusive, exerted no apparent effect on either a four-hour or a twenty-hour culture of pneumococcus. In a dilution of 1:1,000,000 slight temporary inhibition was observed in the case of the four-hour cultures, whereas the twenty-hour culture showed a slight prolongation of the "lag" exhibited by this culture in control serum without optochin. In the next lower dilution, 1:800,000, the four-hour culture showed an unmistakable "lag" or temporary inhibition and the twenty-hour culture showed a decidedly lengthened "lag." In dilution of 1:600,000 there was almost

TABLE 2.—BACTERICIDAL ACTION OF VARIOUS DILUTIONS OF OPTOCHIN IN NORMAL HORSE SERUM

Dilution of Optochin in Normal Horse Serum	Four-Hour Culture Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*					Twenty-Hour Culture Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated†				
	Im- medi- ately	After 1½ Hrs. Inc.	After 4 Hrs. Inc.	After 6½ Hrs. Inc.	After 21 Hrs. Inc.	Im- medi- ately	After 1½ Hrs. Inc.	After 3½ Hrs. Inc.	After 6½ Hrs. Inc.	After 21 Hrs. Inc.
Control serum (no optochin)	321	752	A. C.‡	C.	C.	255	188	184	20,000 Est.	C.
1:5,000,000	309	760	A. C.	C.	C.	281	172	139	20,000 Est.	C.
1:3,000,000	308	770	A. C.	C.	C.	257	162	158	20,000 Est.	C.
1:2,000,000	286	753	A. C.	C.	C.	322	183	150	20,000 Est.	C.
1:1,000,000	291	705	20,000 Est.	C.	C.	284	205	191	15,000 Est.	C.
1:800,000	292	352	1,320	20,000 Est.	C.	266	225	149	164	C.
1:600,000	297	340	383	354	745	305	170	75	37	0
1:400,000	308	301	219	43	0	304	139	49	20	0
1:200,000	306	370	186	30	0	305	78	42	5	0
1:100,000	308	337	93	12	0	243	49	11	1	0
1:50,000	355	207	31	4	0	186	18	9	0	0

\* This culture was not multiplying as rapidly as is usually the case in "young," four-hour cultures. Inoculation: 0.001 c.c. of broth culture of pneumococcus, Group II.

† Inoculation: 0.0001 c.c. of broth culture of pneumococcus, Group II.

‡ In this and the following table A. C. = almost confluent; C = confluent; Est. = estimated.

complete inhibition in the case of the four-hour culture and bactericidal action in that of the twenty-hour culture. In the lower dilutions there was manifested a progressive increase in the bactericidal action of the optochin-containing serum. The experiment shows that variations in the concentration of optochin in normal horse serum are accompanied by corresponding differences in the degree of specific action of the drug on the pneumococcus, and that this method is a reliable one for detecting slight differences in the amount of the drug present in the serum.

The experiment further demonstrates that the individual members of a four-hour broth culture of pneumococcus are more resistant to the action of optochin in horse serum than are the members of the same culture when it has been incubated for twenty hours. This is clear from the more rapid rate at which the pneumococci of the twenty-hour culture are destroyed than that at which the organisms of the four-hour culture succumb, and, in addition, by the observation that optochin in a dilution of 1:600,000 sufficed to exert a bactericidal action on the

older culture, while on the younger it was able to exert an inhibitory effect only. The experiment, therefore, may be said to have borne out the conceptions that were formed as a result of the investigation of the nature of bacterial lag<sup>24</sup> in regard to differences in the resistance of members of a culture of bacteria depending on the age of the culture itself. In such tests as have been carried out in this investigation "young," actively-growing cultures commend themselves for use, since, by their employment, the phenomenon of "lag" is avoided in the controls and the interpretation of results is not confused by its presence.

Not infrequently, when such "young" cultures are used in bactericidal tests, the plates poured after incubation of the serum for one or two hours may show a larger number of colonies than those poured immediately after inoculation; whereas, the plates poured after further incubation (five to twenty hours) of the same specimen of serum, may show a decided decrease in the number of colonies, or even sterility. This temporary initial increase in the number of viable pneumococci may be due to the breaking up of chain forms, which are numerous in "young" broth cultures of pneumococcus; or, it may be due to an appreciable interval of time being required for the bactericidal agent to exert its full effect on the organisms, during which interval some new individuals are formed.

The behavior of the pneumococci in the various dilutions of optochin in normal horse serum and in the serum of patients receiving the drug, has also been followed from the microscopic standpoint. Smears were made at frequent intervals during incubation and stained to show the capsules with Hiss' capsule stain. It was found that pneumococci, after some hours exposure to nonlethal concentrations of optochin in serum, possessed a much larger capsule with a denser outer zone than did the pneumococci grown in serum without optochin. Furthermore, after several hours' exposure the capsules were found to be progressively larger the greater the concentration of optochin to which the organisms had been exposed, provided the concentration was not great enough to exert bactericidal action; frequently, in still greater concentrations, there were vacuoles in the capsular substance, giving it a moth-eaten appearance. The meaning of these phenomena is not clear.

#### DOSAGE OF OPTOCHIN

The patients treated fall into two groups, depending on the route of administration, as follows:

- I. Those receiving all doses by mouth.
- II. Those receiving one or several doses intramuscularly at the outset, and all subsequent doses by mouth.



## I. ORAL ADMINISTRATION ENTIRELY

Twenty-eight patients were treated in this manner. In general, three systems of dosage were employed, which, for the sake of brevity, will be designated A, B and C.

## THE VARIOUS METHODS OF DOSAGE USED; COMPARISON

Dosage A consisted of three doses of 0.5 gm. or 0.45 gm. each, given at regular intervals in the first twenty-four hours, followed in the second twenty-four hours by one dose of 0.45 gm. and seven of 0.15 gm. at regular intervals; thereafter nine or ten doses of 0.15 gm. in every twenty-four hours.

Dosage B consisted of ten doses of 0.15 gm. each, given at regular intervals in every twenty-four hours.

Dosage C consisted, in patients of average size, of a single initial dose of 0.45 gm., followed at regular intervals by single doses of 0.15 gm. each, the total amount of optochin given in twenty-four hours being the same for all periods of twenty-four hours, namely, 1.5 gm. Where patients were below average size and received less than 1.5 gm. in twenty-four hours, the same scheme of separation of doses was adhered to, and their size was proportionately decreased.

In contrasting the results obtained from administering the drug to different patients according to these various methods of dosage, care has been taken to select from the cases studied only those which are comparable as regards (1) amount of drug given per kilogram of body weight per twenty-four hours, and (2) details of bactericidal tests, such as use of young cultures and of inoculations of comparable numbers of pneumococci. Only cases which fulfil these conditions have been selected for the comparison of the various methods of dosage.

*Dosage A.—Several relatively large doses at the outset, followed by numerous smaller ones.*

Two cases in which the patients were treated according to this method will be presented for analysis. Details of the cases follow, together with tables showing the results of a study of the bactericidal power of the serum of each case. In addition, opportunity will be afforded in the first case (Case 1, Hosp. No. 2600) to contrast the inhibitory and bactericidal effect of the serum on "young" (four-hour) actively growing cultures of pneumococci with that on "old" (twenty-hour) cultures of the same strain.

CASE 1 (No. 2600).—C. C., man, waiter, aged 34, weight 65.4 kg.

*Past History.*—Unimportant.

*Present Illness.*—Chill, pain in chest, cough, and bloody sputum three days before admission.

Status on Admission: temperature, 102.2 F.; pulse, 98; respirations, 40;

beginning involvement of right lower lobe; white blood count, 15,000; blood culture sterile; sputum mucopurulent, streaked with blood and containing pneumococcus, Group IV.

Course: On the day following admission the patient was more seriously ill. The temperature rose to 105 F.; the physical signs indicated frank consolidation of the right lower lobe, and blood culture taken at this time showed the presence of pneumococcus, Group IV. Optochin was started on this, the fifth, day of the disease. During the first twenty-four hours three doses of 0.5 gm. each at intervals of eight hours were given; during the second twenty-four hours 0.45 gm. was given in one dose, followed after another interval of eight hours by seven doses of 0.15 gm. each at intervals of two hours; thereafter the drug was given at the rate of ten doses of 0.15 gm. each in twenty-four hours. The patient's condition improved after the optochin was begun, his temperature (rectal) reaching 100 F. on the seventh day, forty-eight hours after optochin was started, and normal on the eighth day. The affected lobe cleared up slowly, although the temperature remained normal. Blood culture, which was positive before treatment was begun, was sterile twenty-four hours after the optochin was started. There was no spread of the pneumonic process to other lobes. Total amount of optochin given, 3.6 gm. No toxic symptoms referable to the optochin were observed. Amount of optochin given per kilo of body weight per twenty-four hours, 0.0229 gm.

TABLE 3.—BACTERICIDAL TEST OF SERUM OF CASE 1 (No. 2600), SHOWING DIFFERENCES OBTAINED WITH "YOUNG" AND "OLD" CULTURES

Time in Hours After Initial Dose of Optochin	Doses of Optochin in Gm. Following Corresponding Test Bleeding	Specimen of Serum Tested	Four-Hour Culture. Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*				Twenty-Hour Culture. No. of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated†		
			Immediately	After 1½ Hrs. Inc.	After 4½ Hrs. Inc.	After 23 Hrs. Inc.	Immediately	After 5 Hrs. Inc.	After 26 Hrs. Inc.
0.0	1 × 0.5	1	951	8,000	A. C.	C.	210	A. C.	C.
8.0	.....	2	867	1,136	20,000 Est.	C.	179	229	50
6.0	.....	3	934	960	10,000	A. C.	277	74	
8.0	1 × 0.5	4	908	2,390	20,000 Est.	C.	190	249	38
11.0	.....	5	876	997	1,820	308	284	4	0
16.0	1 × 0.5	6	970	1,085	2,200	2,000	131	9	0
19.0	.....	7	908	905	745	21	257	2	0
22.0	.....	8	909	700	1,155	180	258	1	0
24.0	1 × 0.45	9	892	888	1,226	16	275	1	0
26.5	.....	10	973	842	1,370	2	53	0	0
29.5	.....	11	988	845	1,565	84	127	0	0
31.75	1 × 0.15	12	885	950	1,271	13	217	0	
34.0	1 × 0.15	13	810	972	1,052	8	176	1	
36.0	1 × 0.15	14	743	908	1,447	33	75	0	0
38.0	8 × 0.15	15	857	970	476	1,500			
46.5	1 × 0.15	16	868	993	1,169	32	31	0	0
48.0	4 × 0.15	17	891	816	1,020	60	67	0	0

\* Inoculation: 0.001 c.c. of broth culture of pneumococcus, Group II.

† Inoculation: 0.00001 c.c. of broth culture of pneumococcus, Group II.

Table 3 shows the difference in the results obtained, depending on whether "young" or "old" cultures are used in such tests. In the case of the twenty-hour-old culture bactericidal action appeared in the second specimen of serum, obtained three hours after the initial dose of optochin; whereas, in the case of the four-hour-old culture bactericidal action was not apparent until several hours later, namely, at eleven hours after the initial dose (Specimen 5). The results of this experiment confirm those obtained in Experiment 1, in which the action of known strengths of optochin in normal horse serum was tested on the same bouillon culture of pneumococcus after four hours' incubation of the same at 37 C., and after twenty hours' incubation, and in which the differences in resistance of the two cultures were demonstrated. This experiment further confirms the preceding one, in that it shows that by using a "young" (four hours at 37 C.) culture in carrying out such tests, it is possible to bring out finer differences in the degree of inhibitory power of the serum tested than is the case if an "old" (twenty hours at 37 C.) culture be employed. For example, this patient (who received 0.0229 gm. of optochin per kilogram of body weight per twenty-four hours), according to the tests with the young culture, acquired a bactericidal action in his serum between eight and eleven hours after the initial dose. Previous to this time temporary inhibition only was present, and close scrutiny of the figures will show that the degree of this inhibitory action varied in the different specimens. Thus, the third specimen of serum, obtained six hours after the initial dose of optochin, showed a greater degree of inhibitory action than did the next specimen obtained two hours later and immediately before the patient received the next dose of optochin. These facts would suggest that at the end of eight hours after the initial dose of 0.5 gm., the blood was already beginning to lose some of the optochin that had been absorbed earlier. Again, the fifth specimen of serum, obtained three hours after the second dose of optochin, showed bactericidal action, whereas the next specimen of serum, obtained eight hours after the second dose and immediately before the third dose, exhibited almost complete inhibition, but no bactericidal action, indicating again variations in bactericidal power within eight hours. On the other hand, with the twenty-hour culture no such clear-cut fluctuations in bactericidal action are observed. It is thus possible, with the aid of young, actively growing cultures, to detect in the serum of patients variations in the degree of specific inhibitory power, and therefore in the concentration of optochin, which would probably not be detected at all, or at least would not be as clearly demonstrated, if twenty-hour cultures were employed instead.

CASE 2 (No. 2705).—B. B., housewife, aged 34, weight 52.8 kg.  
*Past History.*—Unimportant.



*Present Illness.*—Chill, pain in the chest and cough twenty-four hours before admission to the hospital; blood-tinged sputum day of admission.

Status on Admission: temperature, 104.3 F.; pulse, 100; respirations, 34; cyanosis; dulness and suppression of breath sounds over the right lower lobe. Roentgenogram shows shadow in this area. White blood count, 40,000; blood culture sterile; sputum tenacious, hemorrhagic, containing *Pneumococcus mucosus*.

Course: Optochin administration was begun about twenty-four hours after the initial chill. The patient received in the first twenty-four hours three doses of 0.45 gm. each at intervals of eight hours. In the second twenty-four hours she received one dose of 0.45 gm. followed by seven doses of 0.15 gm. each, and in the two succeeding periods of twenty-four hours, nine doses of 0.15 gm. each. In the remaining periods of twenty-four hours, ten doses of 0.15 gm. each were given. The signs of consolidation gradually became more marked over the right lower lobe, and on the sixth day of the disease, five days after commencement of optochin, the physical signs indicated involvement of the left lower lobe, which was borne out by the roentgenogram. At this time a series of intravenous injections of extract of antipneumococcus serum (Group III) was begun, in addition to the optochin. In spite of treatment, the patient from this time on

TABLE 4.—BACTERICIDAL TEST OF SERUM OF CASE 2 (No. 2705)

Time in Hours after Initial Dose of Optochin	Doses of Optochin in Gm. Following Corresponding Test Bleeding	Specimen of Serum Tested	Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*		
			Immediately	After 6 Hours Inc.	After 22½ Hours Inc.
0.0	1 × 0.45	1	1,090	C.	C.
3.0	.....	2	1,024	900	5,600
6.0	.....	3	1,095	984	C.
8.0	1 × 0.45	4	1,100	4,650	C.
11.0	.....	5	1,000	454	2
14.0	.....	6	1,090	764	35
16.0	1 × 0.45	7	1,080	779	968
19.0	.....	8	1,016	305	1
22.0	.....	9	1,050	519	784
24.0	1 × 0.45	10	1,060	631	10
26.0	.....	11	1,026	209	0
32.0	2 × 0.15	12	1,080	366	0
37.0	2 × 0.15	13	1,033	257	1
42.0	2 × 0.15	14	980	345	0
47.0	4 × 0.15	15	1,070	256	0
58.5	3 × 0.15	16	920	93	0
66.5	7 × 0.15	17	1,048	.....	0
85.5	9 × 0.15	18	794	86	0
96.0	3 × 0.15	19	800	56	0
101.0	12 × 0.15	20	1,090	59	2
129.5	7 × 0.15	21	1,033	101	1
154.5	3 × 0.15	22	1,042	61	15

\* Inoculation: Six-hour culture of pneumococcus, Group II.

grew worse, and death occurred on the eighth day of the disease. Immediately after death 40 c.c. of cloudy fluid were aspirated from the pericardial cavity and found to be sterile by cultural tests.

Total amount of optochin given, 9.9 gm. Total amount of extract of antipneumococcus serum given in divided doses, 55 c.c. of 10-times concentrated serum, representing 550 c.c. of whole serum. No toxic symptoms referable to the optochin were observed.

Amount of optochin received per kilogram of body weight per twenty-four hours for each such period was: first period, 0.0255 gm.; second period, 0.028 gm.; third and fourth periods, 0.0255 gm.; thereafter, 0.028 gm.

In Case 2 (Hosp. No. 2705) the results of the serum study were similar to those obtained in the former. Table 4 shows that during the first twenty-four hours of administration of the drug there were fluctuations in the inhibitory and bactericidal power of the serum. Thus, the specimen obtained three hours after the first dose showed greater inhibitory power than did those obtained six and eight hours after the same dose; again, the specimens obtained three and six hours after the second dose both showed definite bactericidal power, whereas the specimen obtained eight hours after that dose showed inhibitory, but not bactericidal, action. After the first twenty-four hours the bactericidal action remained fairly constant throughout. Both this and the preceding case received comparable amounts of optochin per kilogram of body weight per twenty-four hours, and in both instances the drug was given in three large doses in the first twenty-four hours, so that they are quite comparable as regards dosage. They both showed definite fluctuations in the inhibitory and bactericidal power of the serum within the first twenty-four hours, during which time the drug was being given in relatively large doses eight hours apart. It is probable, if the doses were regulated throughout the treatment as in the first twenty-four hours in these two cases, that the fluctuations in the inhibitory and bactericidal power of the serum (and, therefore, in the concentration of the optochin in the circulating blood) would continue, although perhaps be less marked. After a period of treatment, however, owing to the retention or accumulation of optochin within the body, to be referred to later, and to its rapid absorption, such a comparatively large dose as 0.5 gm. might give rise, for a short period after its administration, to a concentration of the drug in the blood injurious to some part of the body, for example, the retina.

As declared in the foregoing, the occurrence of such fluctuations in the concentration of optochin in the blood of patients with lobar pneumonia during treatment with the drug seems undesirable.

*Dosage B.*—*Small equal doses at regular intervals from the outset.*

Several patients were treated according to this method and the details of two of these cases follow, together with the results obtained from a study of the bactericidal power of the serum in each case.

CASE 3 (No. 2639).—P. C., plumber, aged 41, weight 72.8 kg.

*Past History.*—Alcoholic.

*Present Illness.*—Chill, pain in chest and cough the day before admission.

Status on Admission: temperature, 103.5 F.; pulse, 98; respirations, 30; involvement of right middle lobe; roentgenogram shows sharply circumscribed shadow in this area; white blood count, 22,000; blood culture sterile; sputum mucopurulent, blood streaked, containing pneumococcus, Group IV.

Course: Optochin started on second day of disease; optochin given at regular intervals at the rate of 11 doses of 0.15 gm. each in periods of twenty-four hours. Patient's condition improved and on the morning of the fourth day the temperature had reached 100 F., and by the fifth day it was normal. There was no spread of the pneumonic process to any other lobe.

Total amount of optochin given, 4.5 gm. No toxic symptoms referable to the optochin were observed. Amount of optochin given per kilogram of body weight per twenty-four hours, 0.0226 gm.

TABLE 5.—BACTERICIDAL TEST OF SERUM OF CASE 3 (No. 2639)

Time in Hours after Initial Dose of Optochin	Doses of Optochin in Grams Following Corresponding Test Bleeding	Specimen of Serum Tested	Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*		
			Immediately	After 1½ Hours Inc.	After 24 Hours Inc.
0.0	1 × 0.15	1	311	8,830	O.
2.25	1 × 0.15	2	363	2,340	C.
4.5	1 × 0.15	3	320	660	C.
6.75	1 × 0.15	4	376	590	C.
9.0	2 × 0.15	5	334	332	C.
13.5	1 × 0.15	6	333	378	4,600 App.#
16.5	1 × 0.15	7	307	360	79
18.0	1 × 0.15	8	334	352	300
20.25	1 × 0.15	9	324	325	9
22.5	2 × 0.15	10	393	314	6
27.0	2 × 0.15	11	324	343	12
31.5	2 × 0.15	12	320	322	2
36.0	2 × 0.15	13	354	276	2
40.5	2 × 0.15	14	296	375	1
46.0	3 × 0.15	15	260	310	28
51.75	4 × 0.15	16	322	327	0
61.5	.....	17†	298	335	187
65.0	.....	18‡	350	303	10
72.75	.....	19§	352	307	C.
98.5	.....	20¶	297	2,000 Est.	C.

\* Inoculation: 0.001 c.c. of six-hour broth culture of pneumococcus, Group II.

† Two hours after last dose.

‡ Five and one-half hours after last dose.

§ Thirteen and one-fourth hours after last dose.

¶ Thirty-nine hours after last dose.

# App. = approximately.



In Case 3 (Hosp. No. 2639), in which the amount of optochin per kilogram of body weight per twenty-four hours was 0.0226 gm., the bactericidal action appeared within 16.5 hours after the initial dose of optochin, remained practically constant for the remainder of the treatment (with the exception of specimen 8), and disappeared between 5.5 and 13.25 hours after the last dose. Previous to the appearance of the bactericidal action, a progressive increase in the temporary inhibition was observed.

CASE 4 (No. 2659).—M. D., housewife, aged 29, weight 74.5 kg.

*Past History.*—Unimportant.

*Present Illness.*—Chill six days before admission, cough and pain in the chest three days before admission, headache, fever and prostration.

Status on Admission: temperature, 103.6 F.; pulse, 96; respirations, 40; involvement of the left lower lobe; white blood count, 16,000; blood culture sterile; sputum mucopurulent, containing pneumococcus, Group I.

Course: Optochin started on day of admission, sixth day of disease; patient received twelve doses of 0.15 gm. each in twenty-four hours; temperature fell by crisis seven hours after optochin was started; there was no spread of the pneumonic lesion and the affected lobe cleared up gradually.

Total amount of optochin given, 1.8 gm. No toxic symptoms referable to optochin were observed. Amount of optochin given per kilogram of body weight per twenty-four hours, 0.0241 gm.

TABLE 6.—BACTERICIDAL TEST OF SERUM OF CASE 4 (No. 2659)

Time in Hours after Initial Dose of Optochin	Doses of Optochin in Grams Following Corresponding Test Bleeding	Specimen of Serum Tested	Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*			
			Immediately	After 1½ Hours Inc.	After 6 Hours Inc.	After 22 Hours Inc.
0.0	2 × 0.15	1	389	6,000 App.	O.	O.
2.5	.....	2	340	4,000 App.	O.	O.
4.0	1 × 0.15	3	439	4,000 App.	O.	O.
6.0	1 × 0.15	4	378	2,600	O.	O.
8.0	1 × 0.15	5	450	1,800	O.	O.
10.0	1 × 0.15	6	379	890	A. C.	O.
12.0	2 × 0.15	7	443	554	A. C.	O.
16.0	1 × 0.15	8	341	488	A. C.	O.
18.0	2 × 0.15	9	330	443	638	136
22.0	1 × 0.15	10	423	360	217	110
26.0	.....	11†	439	308	357	17
58.0	.....	12‡	279	514	A. C.	O.

\* Inoculation: 0.0001 c.c. of four-hour broth culture of pneumococcus, Group II.

† Four hours after last dose.

‡ Thirty-one hours after last dose.

In Case 4 (Hosp. No. 2659) the amount of optochin given per kilogram of body weight per twenty-four hours was 0.0241 gm. and bactericidal action was manifest within eighteen hours after the initial

dose. Previous to this time there was a steady increase in the temporary inhibitory action of the serum.

On the basis of these two preceding cases, it may be concluded that such a system of dosage gives rise to a progressive increase in the concentration of optochin in the blood stream of the patient, but, as compared with the previous system of dosage, the time of appearance of bactericidal action is somewhat delayed. In order to secure a more rapid appearance of this bactericidal action, and yet to maintain it at a constant level, or at least to avoid such fluctuations as were exhibited by the first system of dosage studied, it would seem advisable to give one comparatively large dose at the outset and follow it with numerous smaller doses at regular intervals, as illustrated in the next section.

*Dosage C.*—An initial comparatively large dose followed at regular intervals by smaller equal ones.

The majority of our patients were treated in this manner, and, from the standpoint of early appearance and constancy of bactericidal action in the serum, the results obtained with this system of dosage were more satisfactory than those with any of the other methods. Below are given the details of four cases so treated, together with the results of a study of the bactericidal power of the serum.

CASE 5 (No. 2640).—D. C., cobbler, aged 46, weight 55.8 kg.

*Past History.*—Pneumonia on the right side seven years previously.

*Present Illness.*—Three days before admission, chill, pain in right side of chest, bloody sputum and general prostration.

Status on Admission: temperature, 104.6 F.; pulse, 110; respirations, 48; involvement of right lower lobe. Roentgenogram shows shadow in this area; white blood count 18,000; blood culture sterile; sputum tenacious, rusty and containing *Pneumococcus mucosus* (Group III).

Course: Optochin started on fourth day of the disease; one dose of 0.45 gm. and seven doses of 0.15 gm. in first twenty-four hours; thereafter ten doses of 0.15 gm. every twenty-four hours. The patient continued to have fever until the ninth day, when the temperature began to fall, reaching 99.8 F. on the tenth day of the disease. There was no spread of the pneumonic process during treatment. Subsequently fibrinous pleurisy developed on the affected side, which was accompanied by moderate fever for several days. Ultimately this condition cleared up also.

Total amount of optochin given, 9.45 gm. No toxic symptoms referable to the drug were observed. Amount of optochin in grams per kilogram of body weight per twenty-four hours, 0.0268.

Table 7 shows that in this case (Case 5, Hosp. No. 2640), in which the amount of optochin given per kilogram of body weight per twenty-four hours was 0.0268 gm., bactericidal action appeared within 5.5 hours after the initial dose of optochin, and after the patient had received one dose of 0.45 gm. and one of 0.15 gm. This action remained constant until more than eight hours after the last dose of optochin had been administered, after which it disappeared. This experiment was repeated with another four-hour-old culture of pneumococcus and similar results were obtained.

TABLE 7.—BACTERICIDAL TEST OF SERUM IN CASE 5 (No. 2640)

Time in Hours after Initial Dose of Optochin	Doses of Optochin in Grams Following Corresponding Test Bleeding	Specimen of Serum Tested	Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*		
			Immediately	After 1½ Hours Inc.	After 24 Hours Inc.
0.0	1 × 0.45 1 × 0.15	1	232	508	C.
5.5	1 × 0.15	2	284	247	1
8.75	1 × 0.15	3	305	282	33
12.0	1 × 0.15	4	278	310	5
14.5	1 × 0.15	5	284	269	5
17.75	1 × 0.15	6	260	280	9
20.75	1 × 0.15	7	314	284	5
23.0	4 × 0.15	8	302	261	2
34.0	3 × 0.15	9	248	253	8
41.0	2 × 0.15	10	296	292	15
46.0	5 × 0.15	11	266	278	0
58.5	4 × 0.15	12	260	308	17
69.5	5 × 0.15	13	211	264	3
91.75	10 × 0.15	14	288	264	0
106.0	10 × 0.15	15	270	265	10
130.5	4 × 0.15	16	269	261	0
139.25	5 × 0.15	17	273	288	13
153.5	1 × 0.15	18	306	255	1
162.0	.....	19†	257	318	2
187.5	.....	20‡	278	252	C.
210.0	.....	21§	249	329	C.

\* Inoculation: 0.0006 c.c. of four-hour broth culture of pneumococcus, Group II.

† Eight hours after last dose.

‡ Thirty-three and one-half hours after last dose.

§ Fifty-six hours after last dose.

CASE 6 (No. 2673).—H. W., business man, aged 39, weight 81.6 kg.

*Past History.*—Alcohol to excess.

*Present Illness.*—Pain in chest, cough, blood-tinged sputum and fever three days before admission.

Status on Admission: temperature, 102.4 F.; pulse, 122; respirations, 34; involvement of right lower lobe; blood culture positive—pneumococcus, Group II; sputum tenacious, blood tinged, containing pneumococcus, Group II; white blood count, 12,800.

Course: Optochin was started on the third day. In the first twenty-four hours an initial dose of 0.5 gm., followed by nine doses of 0.15 gm. each; in second twenty-four hours, thirteen doses of 0.15 gm. each; in third and fourth periods of twenty-four hours, twelve doses of 0.15 gm. each, and in fifth period of twenty-four hours, eleven doses of 0.15 gm. each. On the fourth day of the disease, after twenty-four hours of optochin, blood culture was sterile; on the fifth day physical signs indicated a spread of the pneumonic process to the right upper lobe, and the patient was definitely more ill. Optochin was continued,



and in addition extract of antipneumococcus serum, Type II, was given intravenously. The temperature reached normal on the eighth day.

Total amount of optochin given was 9.5 gm. Total amount of extract of antipneumococcus serum given, in divided doses, was 155 c.c., representing 620 c.c. of whole serum. During administration of optochin the patient developed tinnitus and partial deafness, both of which disappeared after the drug was discontinued.

Total amount of optochin given per twenty-four hours: first twenty-four hours, 1.85 gm.; second twenty-four hours, 1.95 gm.; third and fourth, 1.80 gm.; fifth, 1.65 gm.

Amount of optochin given per kilogram of body weight in the first twenty-four hours, 0.0226 gm.; in the second twenty-four hours, 0.0238 gm.; in the third and fourth twenty-four hours, 0.022 gm.; in the fifth twenty-four hours, 0.0202 gm.

TABLE 8.—BACTERICIDAL TEST OF SERUM IN CASE 6 (No. 2673)

Time in Hours after Initial Dose of Optochin	Doses of Optochin in Grams Following Corresponding Test Bleeding	Specimen of Serum Tested	Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*			
			Immediately	After 1½ Hours Inc.	After 7 Hours Inc.	After 19½ Hours Inc.
0.0	1 × 0.5 1 × 0.15	1	584	7,800	O.	O.
4.0	2 × 0.15	2	550	672	18,000	A. C.
8.0	2 × 0.15	3	604	528	6,200	15,000 Est.
12.0	1 × 0.15	4	537	584	775	232
15.0	1 × 0.15	5	597	588	659	327
17.0	1 × 0.15	6	529	...	994	896
21.0	1 × 0.15	7	530	...	568	175
23.5	6 × 0.15	8	604	...	452	206
36.0†	1 × 0.15	9†	563	579	394	63
37.0‡	1 × 0.15	10‡	613	570	315	25
39.5	2 × 0.15	11	640	495	296	8
42.5	12 × 0.15	12	548	464	282	17
64.25	11 × 0.15	13	624	553	329	9
86.5	12 × 0.15	14	608	557	289	10
112.0	6 × 0.15	15	602	...	386	44
132.0	.....	16§	606	...	466	C.

\* Inoculation: 0.001 c.c. of 6½-hour broth culture of pneumococcus, Group II.

† Immediately before first dose of extract of antipneumococcus serum intravenously.

‡ One hour after first dose of extract of antipneumococcus serum intravenously.

§ Four hours after last dose of optochin.

In Case 6 (Hosp. No. 2673; Table 8) the amount of optochin given per kilogram of body weight in the first twenty-four hours was 0.0226 gm., and bactericidal action was manifest twelve hours after the initial dose of optochin. It persisted as long as the patient continued to receive the drug (except in Specimen 6, where it was replaced by inhibition). Thirty-six hours after the initial dose of optochin, this

patient began to receive extract of antipneumococcus serum intravenously in addition to the optochin; an increase in the bactericidal power of the serum was not demonstrated after this form of treatment was instituted. Since neither the antipneumococcus serum nor the extract thereof possess pneumococidal action, this result was not surprising.

Clinically, this case is of interest because of the fact that, although the patient on the fifth day of the disease had received optochin for thirty-six hours and his blood during twenty-four hours of that time showed a bactericidal action on pneumococci, nevertheless the physical signs indicated that the pneumonic process was spreading at the time and involving another lobe. The blood culture, however, which was positive before the administration of optochin, was sterile after twenty-four hours of optochin treatment.

CASE 7 (No. 2611).—M. B., laborer, aged 60, weight 58.4 kg.

*Past History.*—Unimportant.

*Present Illness.*—Chill thirty-six hours before admission; pain in the chest, cough and blood-tinged sputum twenty-four hours before admission.

Status on Admission: temperature, 102.6 F.; pulse, 112; respirations, 36;

TABLE 9.—BACTERICIDAL TEST OF SERUM IN CASE 7 (No. 2611)

Time in Hours after Initial Dose of Optochin	Doses of Optochin in Grams Following Corresponding Test Bleeding	Specimen of Serum Tested	Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*			
			Immediately	After 1½ Hours Inc.	After 5½ Hours Inc.	After 20 Hours Inc.
0.0	1 × 0.45	1	745	9,100	O.	O.
3.0	1 × 0.15	2	744	2,300	20,000 Est.	O.
6.0	2 × 0.15	3	715	1,550	10,000 Est.	O.
12.0	1 × 0.15	4	757	1,260	4,300	10,000 Est.
15.0	1 × 0.15	5	653	1,080	2,100	594
18.0	1 × 0.15	6	681	930	2,100	455
21.0	2 × 0.15	7	695	1,140	1,700	778
28.5	3 × 0.15	8	672	984	1,380	1,700
33.5	4 × 0.15	9	694	1,210	1,700	198
42.5	1 × 0.15	10	703	1,025	917	41
46.0	1 × 0.15	11†	721	1,104	1,430	306
47.5	1 × 0.15	12‡	715	1,010	1,260	400
50.0	3 × 0.15	13	742	1,120	1,160	580
56.0	3 × 0.15	14§	627	870	1,180	62
66.0	10 × 0.15	15	703	844	550	14

\* Inoculation: 0.001 c.c. of four-hour broth culture of pneumococcus, Group II.

† Immediately before patient received first dose of 10 c.c. of extract of antipneumococcus serum subcutaneously.

‡ One and one-half hours after patient had received 10 c.c. of extract of antipneumococcus serum subcutaneously.

§ Two hours after second dose of 10 c.c. of extract of antipneumococcus serum subcutaneously.

involvement of part of right lower lobe; roentgenogram shows shadow in this area; white blood count, 11,500; blood culture sterile; sputum tenacious, hemorrhagic, containing pneumococcus, Group II.

Course: Optochin started about forty hours after chill. Schedule as follows: dosage expressed in periods of twenty-four hours each; first dose 0.45 gm., followed by seven doses of 0.15 gm.; thereafter ten doses of 0.15 gm. Forty-six hours after the beginning of the administration of optochin treatment, administration of extract of antipneumococcus serum (Group II) was commenced. A total of 40 c.c. of extract was given in divided doses, subcutaneously, representing 220 c.c. of whole serum. The disease ran a protracted course, the temperature remaining elevated and physical signs persisting for thirty days, at which time they began to clear up. For a while it was thought that the patient might have tuberculosis, but this diagnosis was not confirmed. The affected part of the lung ultimately cleared up and the temperature became normal. The condition was thought to be one of delayed resolution. There was no spread of the pneumonic process to any other lobe.

Total amount of optochin given, 5.7 gm. No toxic symptoms referable to the drug were observed. Amount of optochin given per kilogram of body weight per twenty-fours, 0.0256 gm.

In Case 7 (Hosp. No. 2611), in which the amount of optochin given per kilogram of body weight per twenty-four hours was 0.0256 gm., bactericidal action appeared within fifteen hours after the initial dose of optochin, and, with the exception of one specimen (eighth), remained constant while the drug was being administered. This patient, also, received extract of antipneumococcus serum subcutaneously forty-six hours after the optochin was started; no increase in the bactericidal activity of the serum was observed after this treatment was begun. The figures (Table 9) show an initial increase in the number of pneumococci in the first one and one-half hours of incubation; it is followed later in most instances by a diminution. Attention was drawn previously to this phenomenon, which is not frequent.

CASE 8 (No. 2566).—E. Q., man, stenographer, aged 21, weight 67.2 kg.

Past History.—Unimportant.

Present Illness.—Pain in chest, chilly sensations and blood-tinged sputum the day before admission.

Status on Admission: temperature, 102.3 F.; pulse, 96; respirations, 32; dullness with suppression of breath sounds over left lower lobe; pleuritic friction rub in this area; white blood count, 14,200; blood culture sterile; sputum tenacious, blood tinged, containing *Pneumococcus mucosus* (Group III).

Course: Optochin was started on the day of admission, a little more than twenty-four hours after the onset of the disease. Schedule as follows: in first twenty-four hours, first dose 0.45 gm., followed by seven doses of 0.15 gm.; in second twenty-four hours, ten doses of 0.15 gm., and in next 15.5 hours, six doses of 0.15 gm. The temperature remained relatively low and reached normal on the sixth day. The condition of the patient remained excellent; no increase in the extent of the signs present on admission was observed.

Total amount of optochin given, 3.9 gm. The patient complained of ringing in the ears and became slightly deaf, while optochin was being administered. Amount of optochin given per kilogram of body weight per twenty-four hours, 0.0223 gm. (first two periods).

In Case 8 (Hosp. No. 2566) the amount of optochin given in each first and second period of twenty-four hours was 0.0223 gm. Directing



TABLE 10.—BACTERICIDAL TEST OF SERUM IN CASE 8 (No. 2566)

Time in Hours After Initial Dose of Optochin	Doses of Optochin in Gm. Following Corresponding Test Bleeding	Specimen of Serum Tested	Three-Hour Old Culture. Number of Colonies of Pneumococci per 0.5 C.c. Serum When Plated*				Twenty-Four-Hour Old Culture. Number of Colonies of Pneumococci per 0.5 C.c. Serum When Plated†			
			Im-medi-ately	After 1½ Hrs. Inc.	After 5½ Hrs. Inc.	After 22 Hrs. Inc.	Im-medi-ately	After 3½ Hrs. Inc.	After 7½ Hrs. Inc.	After 20½ Hrs. Inc.
0	1 × 0.45	1	117	3,500	C.	C.	148	10,000	C.	
3	1 × 0.15	2	101	202	5,000 Est.	C.	166	158	1,058	5,000 Est.
6	1 × 0.15	3	114	156	4,000 Est.	A. C.	169	109	262	4,000 Est.
9	1 × 0.15	4	110	180	1,680	A. C.	232	62	123	5
12	1 × 0.15	5	137	124	1,150	40	133	57	45	1
15	1 × 0.15	6	92	115	662	26	204	63	46	0
18	1 × 0.15	7	90	131	770	A. C.	174	62	64	0
21	1 × 0.15	8	127	154	531	10	199	53	42	0
24	7 × 0.15	9	115	128	661	872	166	65	52	1
42	5 × 0.15	10	106	117	400	900	210	44	30	4
54	4 × 0.15	11	106	630	A. C.	....	168	222	A. C.	C.
‡	.....	12‡	116	1,740	C.	....	195	5,000	C.	C.

\* Inoculation: Broth culture of pneumococcus, Group II.

† Inoculation: Broth culture of pneumococcus, Group II.

‡ One and one-fourth hours after last dose.

attention first to the test made with the three-hour culture, it is seen that bactericidal action made its appearance within twelve hours after the initial dose of optochin (Specimen 5); it was not constantly present, however, as Specimen 7 of serum obtained eighteen hours after the first dose showed only temporary inhibition; Specimens 9 and 10, obtained twenty-four and forty-two hours, respectively, after the commencement of the drug, showed temporary inhibition likewise. One explanation which may be offered to account for the substitution of inhibitory for bactericidal action in these specimens is that the time elapsing between some of the individual doses about these periods was almost four hours (doses 6 and 7, and doses 12, 13 and 14). Such intervals between doses of 0.15 gm. may be too great to maintain the drug at a constant concentration in the circulating blood, and evidence will be presented later in support of this contention. Specimen of serum 11, obtained fifty-four hours after the initial dose of optochin and one and three-quarters hours after the immediately preceding dose was given, showed no bactericidal action and comparatively slight temporary inhibition, while that obtained one and a quarter hours after the last dose showed almost no inhibition whatever. This late disappearance of bactericidal action may possibly be accounted for, in addition, by the fact that during this time the patient was receiving

the drug at the rate of 1.23 gm. in twenty-four hours, instead of 1.5 gm., which factor would alter materially the body-weight relationship, making the amount of optochin received per kilogram of body weight per twenty-four hours only 0.018 gm. As judged by the same culture, twenty hours old, bactericidal action made its appearance nine hours after the initial dose of the drug (Specimen 4), that is, three hours earlier than when the four-hour culture was used for the test; moreover, it was constantly maintained, except in Specimens 11 and 12, in which it was replaced by temporary inhibition, as when the four-hour culture was used.

This test shows, again, the contrast between the results obtained with "old" and "young" cultures. The twenty-hour culture did not show the same fluctuation in bactericidal action as did the four-hour culture, and this action was observed three hours earlier in the case of the former.

The results obtained from a study of these three methods of dosage, with reference to time of appearance of bactericidal action, are summarized in Table 11.

TABLE 11.—COMPARISON OF METHODS OF DOSAGE WITH REFERENCE TO THE TIME OF APPEARANCE OF BACTERICIDAL ACTION

Method of Dosage	Case Number	Amount of Optochin per Kilo of Body Weight in First 24 Hours, Gm.	Time of Appearance of Bactericidal Action, Hrs.
A	1 (2600)	0.0230	11.0
	2 (2705)	0.0255	11.0
	Average.....	0.0242	11.0
B	3 (2639)	0.0226	16.5
	4 (2659)	0.0241	18.0
	Average.....	0.0233	17.25
C	5 (2640)	0.0268	5.5
	6 (2673)	0.0226	12.0
	7 (2611)	0.0256	15.0
	8 (2566)	0.0223	12.0
	Average.....	0.0243	11.1

An analysis of Table 11 shows that the cases are quite comparable as regards the relationship of amount of optochin given in twenty-four hours to body weight of the patients. It shows, further, that, with Dosage A, the average time of appearance of bactericidal action was

eleven hours after the initial dose of optochin, and, as was seen from the protocols, that, under this system of dosage, the inhibitory and bactericidal action was inconstant during the first twenty-four hours. In the case of Dosage B, the average time of appearance of bactericidal action was 17.25 hours. With Dosage C the average time of appearance of the same was 11.1 hours, and (with the exception of Case 8, Hosp. No. 2566) it remained constant. On a basis of these facts it seems justifiable to conclude that the third method of dosage, namely, Dosage C (p. 627), is the most satisfactory one to adopt in order to obtain a bactericidal action in the serum in the shortest possible time, and to maintain it at a constant level. This conclusion is supported by further cases, given in detail later.

#### RELATIONSHIP BETWEEN BODY WEIGHT OF PATIENT AND APPEARANCE OF BACTERICIDAL ACTION IN THE SERUM

As stated previously, if the rates of absorption and elimination of optochin are comparable in different cases, the concentration of the drug in the blood stream might be expected to bear some relationship to the body weight of the patient; that is to say, the concentration of optochin should be greater in those cases which receive a larger quantity of the drug per kilogram of body weight than in those which receive a lesser amount. In view of these considerations, it is of interest to study the occurrence of bactericidal action in the serum with reference to the relationship to body weight of the amount of optochin given per twenty-four hours in the cases which are strictly comparable as regards method of dosage. We shall, therefore, with this idea in mind, take up a consideration of those cases in which the patients were treated according to that system of dosage which appears to be the optimum as regards spacing of individual doses and time of appearance of bactericidal action. On the basis of the results given in Table 9 it would seem that the optimum method is represented by Dosage C (p. 627), in which the patient is given a single comparatively large initial dose, followed by smaller ones at regular intervals. Sixteen patients were treated by mouth according to this method of dosage, and of these, eleven have been selected for comparison; that is, only those cases that are strictly comparable as regards spacing of doses and detail of bactericidal tests, such as age of culture and size of inoculum. The weights of these patients varied from 39.1 to 81.6 kg., and the total amount of optochin given in twenty-four hours, from 1.0 to 1.85 gm.

With reference to the relationship between the body weight of the patient and the amount of optochin received per twenty-four hours, it is possible to group the cases as follows:

1. Where the patients were of average size and the amount of optochin given to each in twenty-four hours was 1.5 gm., so that the amount



of optochin per kilogram of body weight per twenty-four hours was from 0.022 to 0.0268 gm. Three cases, 8, 7 and 5 (Hosp. Nos. 2566, 2611 and 2640) illustrate this condition, and the details of the serologic study of each have already been given (see section on dosage, Tables 10, 9 and 7). These cases show that under this system of dosage and with the relationship of body weight to amount of optochin such that the patients received 0.0223 to 0.0268 gm. per kilogram per twenty-four hours, bactericidal action was manifest in less than fifteen hours in each instance.

2. Where the patients were above average size and the total amount of optochin (1.5 gm.) given in twenty-four hours was such that the patient received less than 0.022 gm. per kilogram per twenty-four hours. Three patients were so treated; in one of them (Case 9, Hosp. No. 2581) it was impossible to demonstrate any bactericidal action at all, and in the remaining two (Hosp. Nos. 2568 and 2634) the bactericidal action was considerably delayed. In Hosp. No. 2568 the amount of optochin given per kilogram of body weight per twenty-four hours was 0.0201 gm., and bactericidal action did not appear until between sixty-eight and ninety-two hours after the first dose; in Hosp. No. 2634 the amount of optochin given per kilogram of body weight per twenty-four hours was 0.0213 gm. and bactericidal action took twenty-six hours to appear. Below are given the details of Case 9 (Hosp. No. 2581) with the results of a study of the serum of the same (Table 12).

CASE 9 (No. 2581).—T. B., watchman, aged 35, weight 80 kg.

*Past History.*—Unimportant.

*Present Illness.*—Chill and vomiting thirty-six hours before admission; pain in chest, cough and bloody sputum twenty-four hours before admission.

Status on Admission: temperature, 103.8 F.; pulse, 116; respirations, 36; involvement of right lower lobe; white blood count, 23,000; blood culture sterile; sputum tenacious, hemorrhagic, containing pneumococcus, Group II.

Course: Optochin was started by mouth on the third day of the disease; initial dose of 0.5 gm. and seven doses of 0.15 gm. each in first twenty-four hours; thereafter ten doses of 0.15 gm. in every subsequent period of twenty-four hours. The patient became steadily worse, and on the fifth day, after two and one-half days of administration, the drug was discontinued, and the patient was given extract of antipneumococcus serum, Type II, subcutaneously. Temperature began to fall on the tenth day of the disease and reached 99.8 F. on the eleventh day. In this case both the optochin and the extract of antipneumococcus serum were discontinued, as clinical observation seemed to indicate that the course of the disease was not being influenced by either.

Total amount of optochin given, 3.8 gm. Total amount of extract of antipneumococcus serum given was 106 c.c., in divided doses, representing 583 c.c. of whole serum. No toxic symptoms referable to the optochin were observed. Amount of optochin per kilo of body weight per twenty-four hours was 0.0187 gm.

Table 12 shows that the patient (Hosp. No. 2581) did not possess a bactericidal power in the blood serum at any time during the course of administration of optochin, the figures only showing temporary

TABLE 12.—BACTERICIDAL TEST OF SERUM IN CASE 9 (No. 2581)

Time in Hours after Initial Dose of Optochin	Doses of Optochin in Grams Following Corresponding Test Bleeding	Specimen of Serum Tested	Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*		
			Immediately	After 7½ Hours Inc.	After 15½ Hours Inc.
0.0	1 × 0.5	1	1,800	A. C.	C.
3.0	1 × 0.15	2	1,404	T. N.†	C.
6.0	1 × 0.15	3	1,356	3,500 App.	C.
9.0	1 × 0.15	4	1,078	2,600 App.	C.
12.0	1 × 0.15	5	1,580	1,620	0
15.0	1 × 0.15	6	1,640	988	C.
18.0	1 × 0.15	7	1,528	768	C.
21.0	1 × 0.15	8	1,412	1,612	C.
24.0	2 × 0.15	9	1,484	1,416	C.
30.0	2 × 0.15	10	1,436	1,774	A. C.
36.0	3 × 0.15	11	1,470	1,900	C.
42.0	6 × 0.15	12	1,330	1,488	A. C.
58.5	2 × 0.15	18	1,354	1,456	A. C.

\* Inoculation: 0.00001 c.c. of an eighteen-hour broth culture of pneumococcus, Group I.

† T. N. = too numerous to count.

inhibition. The amount of optochin given per twenty-four hours per kilogram of body weight was 0.0187 gm. Clinically, the course of the disease seemed to be entirely uninfluenced by the drug. At this point it is desirable to draw attention to Case 8 (Hosp. No. 2566, Table 10), in which bactericidal action was demonstrable within twelve hours after the administration of the drug was begun, during which time optochin was being given on a basis of 0.0223 gm. per kilogram of body weight per twenty-four hours. When, toward the end of the course of administration, the amount of drug was diminished, so that the quantity given per kilogram of body weight per twenty-four hours amounted to 0.018 gm., no bactericidal action, and only a slight degree of temporary inhibition, was observed.

3. Where the patient was above average size (81.6 kg.), the amount of the drug (1.85 gm. to 1.95 gm.) given per twenty-four hours was such that the patient received from 0.0226 gm. to 0.0238 gm. of optochin per kilogram per twenty-four hours (first forty-eight hours). Such a condition was illustrated by one case (Case 6, Hosp. No. 2673), which has already been reported in detail. (See section on Dosage, Table 8.) In this case bactericidal action was manifest within twelve hours after the initial dose.

4. Where the patients were below average size and the total amount of the drug given per twenty-four hours was less than 1.5 gm., but the

quantity given per kilogram of body weight per twenty-four hours was from 0.025 to 0.0285 gm. (a relationship which in an adult of average size should, as has been seen, insure bactericidal action in the serum within twenty-four hours). Two patients were so treated. The details of the results of the bactericidal tests in these two cases are given (Tables 13 and 14).

CASE 10 (No. 2675).—J. S., schoolboy, aged 15, weight 39.1 kg.

*Past History.*—Unimportant.

*Present Illness.*—Pain in chest, cough and fever two days before admission.

Status on Admission: temperature, 104.2 F.; pulse, 112; respirations, 32; involvement of left lower lobe; white blood count, 38,000; blood culture sterile; sputum tenacious, mucopurulent, containing pneumococcus, Group IV.

Course: Optochin started on fourth day of disease; patient received during first twenty-four hours an initial dose of 0.3 gm., followed by seven doses of 0.1 gm. Within twenty-four hours after the optochin was started, crisis set in and the temperature was normal on the fifth day. There was no spread of the pneumonic process to any other lobe.

Total amount of optochin given, 1 gm. No toxic symptoms referable to the drug were observed. Amount of optochin per kilo of body weight per twenty-four hours, 0.0255 gm.

TABLE 13.—BACTERICIDAL TEST OF SERUM IN CASE 10 (No. 2675)

Time in Hours after Initial Dose of Optochin	Doses of Optochin in Grams Following Corresponding Test Bleeding	Specimen of Serum Tested	Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*			
			Immediately	After 1½ Hours Inc.	After 6 Hours Inc.	After 23½ Hours Inc.
0.0	1 × 0.3	1	680	10,100	0.	0.
3.5	1 × 0.1	2	628	762	91,000	0.
6.5	1 × 0.1	3	641	627	6,000	0.
9.5	1 × 0.1	4	623	693	2,500	0.
12.5	1 × 0.1	5	810	688	1,700	10,000 Est.
15.5	1 × 0.1	6	676	627	848	417
18.5	1 × 0.1	7	644	680	567	35
21.5	1 × 0.1	8	665	746	520	10
24.5	.....	9	612	500	332	40

\* Inoculation: 0.001 c.c. of four-hour broth culture of pneumococcus, Group II.

CASE 11 (No. 2626).—O. H., schoolboy, aged 15, weight 42.2 kg.

*Past History.*—Chronic otitis media.

*Present Illness.*—Chill, fever, cough and blood-tinged sputum four days before admission.

Status on Admission: temperature, 104 F.; pulse, 104; respirations, 44; bilateral otitis media with perforation of both drums and partial deafness; involvement of left lower lobe; white blood count, 15,000; blood culture sterile; sputum mucoid, blood tinged, containing pneumococcus, Group II.

Course: Optochin started on the sixth day of the disease; patient given 0.3 gm., then nine doses of 0.1 gm. each, making 1.2 gm. in first twenty-four hours; then twelve doses of 0.1 gm. each in second twenty-four hours, and eight doses of



0.15 gm. each in succeeding periods of twenty-four hours. Temperature showed morning remissions on eighth, ninth and tenth days and reached normal on the eleventh day of the disease. There was no spread of the pneumonic process to any other lobe. Lung cleared up slowly.

Total amount of optochin given, 5.65 gm. During the administration of the drug the patient became almost completely deaf, but this condition receded to that present on admission when the optochin was discontinued. Amount of optochin per kilo of body weight per twenty-four hours, 0.0284 gm.

TABLE 14.—BACTERICIDAL TEST OF SERUM OF CASE 11 (No. 2626)

Time in Hours after Initial Dose of Optochin	Doses of Optochin in Grams Following Corresponding Test Bleeding	Specimen of Serum Tested	Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*		
			Immediately	After 1½ Hours Inc.	After 23 Hours Inc.
0.0	1 × 0.3	1	690	3,870	O.
2.5	1 × 0.1	2	714	723	O.
5.0	1 × 0.1	3	719	792	O.
7.5	1 × 0.1	4	928	1,034	O.
10.0	3 × 0.1	5	726	767	O.
17.75	3 × 0.1	6	690	767	A. O.
24.5	3 × 0.1	7	796	701	1
31.0	6 × 0.1	8	700	703	1
42.75	4 × 0.1	9	720	650	4
52.75	4 × 0.1	10	648	705	12
72.75	11 × 0.1	11	677	620	3

\* Inoculation: 0.0005 c.c. of four-hour broth culture of pneumococcus, Group II.

A study of Tables 13 and 14 shows that in Case 10 (Hosp. No. 2675), where the amount of optochin per kilogram of body weight per twenty-four hours was 0.0255 gm., bactericidal action was present within 15.5 hours after the initial dose, although this patient received a total of only 1.0 gm. in twenty-four hours; and that in Case 11 (Hosp. No. 2626), where the amount of optochin received per kilogram per twenty-four hours was 0.0284 gm., the serum acquired a bactericidal action between 17.5 and 24.5 hours after the initial dose, although the total amount of optochin given in twenty-four hours was only 1.2 gm.

5. Finally, where patients are of average size but the quantity of drug is increased over that usually given, so that the amount received per kilogram per twenty-four hours is more than 0.028 gm. Two cases so treated are available for study. One of these (Hosp. No. 2638, Case 17) is recorded in detail later in connection with another question, and reference to Table 22 will show that, in this case, where the amount of optochin per kilogram of body weight per twenty-four hours was 0.031 gm., bactericidal action was present within eleven hours after the initial dose. The details of the other case follow immediately (Table 15).

CASE 12 (No. 2711).—A. S., tinsmith, aged 21, weight 57.4 kg.

*Past History.*—Unimportant, except that he drank beer freely.

*Present Illness.*—Chill and vomiting two days before admission. Cough and blood-tinged sputum day before admission.

Status on Admission: temperature, 104.8 F.; pulse, 104; respirations, 36. Beginning involvement of left lower lobe; white blood count, 20,000; blood culture sterile; sputum tenacious, hemorrhagic, containing pneumococcus, Group I.

Course: On day following admission the patient was more toxic and physical signs at this time indicated beginning involvement of the left upper lobe, which was borne out by examination the next day. Optochin was started on the fourth day of the disease. A blood culture taken immediately before commencement of the optochin showed the presence of three colonies of pneumococcus, Group I, per cubic centimeter of blood. The patient received in the first twenty-four hours one dose of 0.45 gm., followed by eight doses of 0.15 gm. each at regular intervals. Thereafter he received ten doses of 0.15 gm. each, at regular intervals, in periods of twenty-four hours each. On the fifth day of the disease, twenty-four hours after the commencement of the optochin, the patient received the first of four intravenous injections of antipneumococcus serum (Group I), 75.0 c.c. each, given at intervals of nine to twelve hours. The temperature remained approximately constant until the eighth day of the disease, when it fell below 100 F. The lungs cleared slowly.

Total amount of optochin given, 4.05 gm. Total amount of antipneumococcus serum (Group I), 300 c.c. No toxic symptoms referable to the optochin were observed. Amount of optochin per kilo of body weight, 0.0287 gm. first twenty-four hours; thereafter, 0.026 per twenty-four hours.

TABLE 15.—BACTERICIDAL TEST OF SERUM OF CASE 12 (HOSP. No. 2711)

Time in Hours after Initial Dose of Optochin	Doses of Optochin in Grams Following Corresponding Test Bleeding	Specimen of Serum Tested	Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*			
			Immediately	After 1½ Hours Inc.	After 5 Hours Inc.	After 21 Hours Inc.
0.0	1 × 0.45	1	188	390	2,000	C.
2.0	1 × 0.15	2	108	165	159	79
4.0	1 × 0.15	3	182	162	158	4
6.0	1 × 0.15	4	170	188	109	4
9.0	1 × 0.15	5	153	142	251	39
12.0	1 × 0.15	6	135	161	139	8
15.0	1 × 0.15	7	163	184	124	66
18.0	1 × 0.15	8	139	178	151	199
21.0	1 × 0.15	9	141	97	160	706
23.0†	.....	10	122	137	201	168
24.0	4 × 0.15	11	131	161	180	1,015
32.5†	15 × 0.15	12	101	170	110	14

\* Inoculation: 0.001 c.c. of four-hour broth culture of pneumococcus, Group II.

† Indicates that at this point the patient was given 75 c.c. of antipneumococcus serum (Group I) intravenously immediately after the corresponding test bleeding.

In Case 12 (Hosp. No. 2711) the amount of optochin per kilogram of body weight given in the first twenty-four hours was 0.0287 gm. and bactericidal action was manifest within two to four hours after the

initial dose. The culture used in this test was not growing very actively; this was afterwards discovered to be due to the fact that the acidity of the broth used was too high. After the patient had received the drug for fifteen hours, complete inhibition rather than bactericidal action was present (Specimens 8 to 11, inclusive). The reason for this decrease in the bactericidal activity of the patient's serum is not apparent; however, bactericidal action was again manifest within 32.5 hours after the first dose of optochin (Specimen 12). It should be noted that a blood culture taken twenty-four hours after the initial dose of optochin showed three colonies of pneumococcus, Group I, in 6 c.c. of blood; reference to Table 15 shows that at this time bactericidal action had been supplanted by mere inhibitory action.

The eleven cases that have been selected for a study of the influence of the factor of body weight on the appearance of bactericidal action are summarized in Table 16, where the cases are arranged in the order of the amount of optochin given per kilogram of body weight per twenty-four hours (first twenty-four hours).

TABLE 16.—RELATIONSHIP BETWEEN AMOUNT OF OPTOCHIN PER KILOGRAM OF BODY WEIGHT PER TWENTY-FOUR HOURS TO TIME OF APPEARANCE OF BACTERICIDAL ACTION

Hospital and Case No.	Weight, Kg.	Total Amount Optochin in First 24 Hours, Gm.	Amount of Optochin per Kilo Body Weight in First 24 Hours, Gm.	Time of Appearance of Bactericidal Action, Hrs.
9 (2581)	80	1.5	0.0187	No bactericidal action
13 (2568)	74.4	1.5	0.0201	68-92
(2634)	70.3	1.5	0.0213	26
8 (2566)	67.2	1.5	0.0223	12
6 (2673)	81.6	1.86	0.0226	12
7 (2611)	58.4	1.5	0.0256	15
10 (2675)	89.1	1.0	0.0255	15.5
5 (2640)	55.8	1.5	0.0268	5.5
11 (2626)	42.2	1.2	0.0284	17.5-24.5
12 (2711)	57.4	1.65	0.0287	4
17 (2698)	42.8	1.35	0.0315	11

A study of Table 16 shows that those patients who received less than 0.022 gm. of optochin per kilogram of body weight per twenty-four hours either did not acquire in their serum any bactericidal action, or else its appearance was delayed more than twenty-four hours from the commencement of the optochin treatment; whereas, the serum of those patients who received more than 0.022 gm. of optochin per kilo-



gram of body weight per twenty-four hours in no instance failed to show definite bactericidal properties. Furthermore, in those patients receiving more than 0.022 gm. of the drug per kilogram of body weight per twenty-four hours, the average time of appearance of the bactericidal action in the serum was 12.4 hours after the giving of the first dose.

An analysis of all our cases in which the patients were treated by mouth, irrespective of the spacing and size of individual doses, shows that in no instance did bactericidal action fail to be manifest when the patients received as much as 0.024 gm. of optochin per kilogram of body weight per twenty-four hours, with one exception (Hosp. No. 2689), where too great an interval—eight hours—elapsed between the first and second doses (see Table 31). We feel justified in concluding, therefore, that in administering optochin to human beings by mouth it is essential that the body weight of the patient be taken into consideration if the production of bactericidal action in the serum is to be insured. Individual variations in absorption and elimination are to be expected, as will be indicated later, but in the cases studied no great differences in these factors have been observed; indeed, it is rather surprising that the relationship between body weight and amount of optochin given per twenty-four hours bears such a constant relationship to the occurrence of bactericidal action in the patient's serum.

## II. COMBINED INTRAMUSCULAR AND ORAL ADMINISTRATION

Table 16 shows that when optochin is given to human beings by mouth, in accordance with a definite system of regulation of doses and in such quantity that the patients receive at least 0.022 gm. of the drug per kilogram per twenty-four hours, the serum of such persons rapidly acquires (average 12.4 hours) a definite bactericidal action for the pneumococcus *in vitro*. It would seem that such an effect might be secured within a shorter space of time if the first few doses were given intramuscularly. Optochin hydrochlorid is soluble in distilled water, less soluble in physiologic salt solution. When given subcutaneously it may lead to painful infiltration. In one patient in whom it was injected too superficially, a slough was produced at the site of injection. When given intramuscularly it does not seem to be irritant.

Three patients (Hosp. Nos. 2658, 2593 and 2604) received one or more doses by the intramuscular route at the outset of the treatment, and subsequent doses by mouth. In each case the size and spacing of the individual doses conformed to what we believe to be the optimum method, and the amount of the drug given was such that in the first twenty-four hours each patient received 0.022 gm. or more per kilogram

of body weight, an amount which, judging from the results obtained from oral administration, should give rise to the production of a bactericidal action in the patient's serum within twenty-four hours.

CASE 13 (No. 2658).—J. E., manager, aged 29, weight 66.6 kg.

*Past History.*—Unimportant.

*Present Illness.*—Chill, pain in chest, cough, blood-tinged sputum day before admission.

Status on Admission: temperature, 102.5 F.; pulse, 120; respirations, 28; involvement of right lower lobe; white blood count, 47,000; sputum tenacious, blood tinged, containing pneumococcus, Group II A; blood culture sterile.

Course: Optochin started day of admission, second day of disease. Patient was given 0.5 gm. intramuscularly, then four doses of 0.25 gm. intramuscularly, all dissolved in physiologic salt solution, making 1.5 gm. in the first twenty-four hours; then ten doses of 0.15 gm. each in powder form by mouth during the succeeding periods of twenty-four hours. The temperature became normal on the fifth day of the disease and there was no spread of the pneumonic process to any other lobe.

Total amount of optochin given, 3.9 gm. No toxic symptoms referable to the optochin were observed. Amount of optochin per kilo of body weight per twenty-four hours was 0.0225 gm.

TABLE 17.—BACTERICIDAL TEST OF SERUM IN CASE 13 (No. 2658)

Time in Hours after Initial Dose of Optochin	Doses of Optochin in Grams Following Corresponding Test Bleeding	Specimen of Serum Tested	Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*			
			Immediately	After 1½ Hours Inc.	After 6 Hours Inc.	After 20 Hours Inc.
0.0	1 × 0.5 †	1	888	13,000	5,000,000 Est.	O.
2.0	.....	2	352	901	2,000,000 Est.	O.
4.0	.....	3	402	548	500,000 Est.	O.
6.0	.....	4	850	495	500,000 Est.	O.
8.0	1 × 0.25 †	5	342	621	A. C.	O.
10.0	.....	6	878	834	10,000 Est.	O.
12.0	1 × 0.25 †	7	367	888	10,000 Est.	O.
14.0	.....	8	809	837	1,036	5,000
16.5	1 × 0.25 †	9	364	423	2,000	2,200
18.0	.....	10	419	462	968	191
20.0	1 × 0.25 † 2 × 0.15 ‡	11	445	485	970	1,124
28.5	3 × 0.15 ‡	12	402	472	404	9
36.0	2 × 0.15 ‡	13	877	478	816	10
40.5	3 × 0.15 ‡	14	402	473	290	1
47.5	2 × 0.15 ‡	15	350	426	341	3
52.0	4 × 0.15 ‡	16	430	497	300	6
		17	438	878	301	

\* Inoculation: 0.001 c.c. of six-hour broth culture of pneumococcus, Group II.

† Intramuscularly.      ‡ Orally.

In Case 13 (Hosp. No. 2658), in which the amount of optochin per kilogram of body weight per twenty-four hours was 0.0225 gm., bactericidal action was present in only one of eleven specimens of serum obtained during twenty-four hours of intramuscular administration of the drug, namely, in specimen 10 (eighteen hours after the commencement of the treatment); it was no longer present in the specimen taken (Specimen 11) two hours afterwards. Indeed, with the one exception of Specimen 10, bactericidal action only became manifest after oral administration had been begun; in other words, between twenty and twenty-eight and one-half hours after the initial dose. Previous to this, and with the exception of Specimen 10, the serum only showed temporary inhibition.

Two patients received initial doses of the drug, dissolved in distilled water, intramuscularly, all subsequent doses being given by mouth. The details of these cases follow.

CASE 14 (No. 2593).—F. B., salesman, aged 37, weight 53.8 kg.

*Past History.*—Unimportant.

*Present Illness.*—Cold for a week before admission. Pain in chest day before admission. No chill or other symptoms.

*Status on Admission:* temperature, 102.4 F.; pulse, 98; respirations, 36; involvement of right lower lobe; white blood count, 12,500; blood culture sterile; sputum tenacious, rusty, containing *Pneumococcus mucosus* (Group III).

*Course:* Optochin started on second day of disease. A first dose of 0.4 gm. dissolved in 8 c.c. of distilled water was given intramuscularly; all subsequent doses were given by mouth. Following the first dose the patient received seven doses of 0.15 gm. each, so spaced that at the end of the first twenty-four hours he had received 1.45 gm., including the dose given intramuscularly. On account of ringing in the ears, the dosage was reduced in the second twenty-four hours, but was subsequently increased. The schedule was as follows: Dosage expressed in periods of twenty-four hours each, beginning with the second twenty-four hours; six doses of 0.15 gm., nine doses of 0.15 gm., eight doses of 0.15 gm. and ten doses of 0.15 gm. for the remainder of the treatment. The condition of the patient grew slowly but steadily worse. The involved area increased in extent until the entire right lung was affected. On the ninth day the left upper lobe showed signs of involvement, and death took place on this day. Blood culture taken eight hours before death showed thirteen colonies of *Pneumococcus mucosus* per cubic centimeter of blood. Optochin had to be discontinued thirty hours before death, owing to the inability of the patient to swallow. The patient developed ringing in the ears and partial deafness while receiving the drug.

Total amount of optochin given, 7.9 gm. Amount of optochin per kilo of body weight, first twenty-four hours, 0.0268 gm.; second twenty-four hours, 0.0167 gm.; third twenty-four hours, 0.025 gm.; fourth twenty-four hours, 0.0223 gm.; thereafter per twenty-four hours, 0.0278 gm.

In Case 14 (Hosp. No. 2593), in which the quantity of drug administered within the first twenty-four hours was 1.45 gm. (of which the first dose of 0.4 gm. was given intramuscularly), equivalent to 0.0269 gm. per kilogram of body weight, no bactericidal action was manifest within the first twenty-four hours; nor was there any considerable inhibition seen in the four hours following the first dose,



which was given intramuscularly. During the second twenty-four hours of administration the patient showed no bactericidal action, but only temporary inhibition, as might be expected, since during this period only 0.9 gm. was given, which means 0.0167 gm. per kilogram; nor did either of the two specimens tested after this time show more than complete inhibition. As no further specimen was tested after No. 13, it is impossible to state whether or not bactericidal action appeared in the serum of this patient during the remaining days of the administration of the drug. In spite of the specific treatment the pneumonic process was not controlled and the patient died.

TABLE 18.—BACTERICIDAL TEST OF SERUM OF CASE 14 (No. 2593)

Time in Hours after Initial Dose of Optochin	Doses of Optochin in Grams Following Corresponding Test Bleeding	Specimen of Serum Tested	Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*		
			Immediately	After 1½ Hours Inc.	After 22 Hours Inc.
0.0	1 × 0.4†	1	339	3,800	O.
1.5	.....	2	421	2,000	O.
2.5	.....	3	380	1,543	O.
4.0	1 × 0.15‡	4	400	1,264	O.
7.0	1 × 0.15‡	5	438	978	O.
10.0	2 × 0.3‡	6	409	552	O.
16.0	2 × 0.3‡	7	398	570	904
22.0	1 × 0.15‡	8	422	732	1,000 App.
27.0	3 × 0.15‡	9	470	688	2,000 App.
39.0	3 × 0.15‡	10	440	498	2,000 App.
48.0	1 × 0.15‡	11	407	484	A. C.
51.0	5 × 0.15‡	12	337	464	3,000 App.
63.75	10 × 0.15‡	13	354	470	650

\* Culture used for inoculation: 0.001 c.c. of four-hour culture of pneumococcus, Group II.

† Intramuscularly.

‡ Orally.

CASE 15 (No. 2604).—D. del V., elevator operator, aged 20, weight 55.4 kg.

*Past History.*—Unimportant.

*Present Illness.*—Chill, fever, cough, pain in chest and bloody sputum two days before admission.

Status on Admission: temperature, 104.6 F.; pulse, 138; respirations, 36. Mental confusion; complete consolidation of left lower lobe; white blood count, 13,000; blood culture sterile; sputum tenacious, rusty and containing pneumococcus of Group II A.

Course: Optochin was started on fourth day of disease. Patient was given a first dose of 0.45 gm. dissolved in 9 c.c. of distilled water intramuscularly; all subsequent doses were given by mouth. Following the first dose the drug was given in divided doses, so that at the end of the first twenty-four hours he had received seven doses of 0.15 gm. in addition to the first dose of 0.45 gm., making 1.5 gm. in all. Thereafter the drug was given at the rate of 10 doses of 0.15 gm. in twenty-four hours. When the patient had received optochin for forty-eight

hours he was given 2 c.c. of extract of antipneumococcus serum, Type II, intravenously, and later 20 c.c. of the same serum in two doses subcutaneously. Temperature reached normal on the seventh day of the disease after three days' administration of the drug.

Total amount of optochin given, 4.5 gm. Total amount of extract of antipneumococcus serum (Group II) given, 22 c.c., representing 111 c.c. of whole serum. No toxic symptoms referable to the drug were observed. Amount of optochin per kilo of body weight per twenty-four hours, 0.0270 gm.

TABLE 19.—BACTERICIDAL TEST OF SERUM OF CASE 15 (No. 2604)

Time in Hours after Initial Dose of Optochin	Doses of Optochin in Grams Following Corresponding Test Bleeding	Specimen of Serum Tested	Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*			
			Immediately	After 1½ Hours Inc.	After 5½ Hours Inc.	After 24 Hours Inc.
0.0	1 × 0.45†	1	476	11,800	O.	O.
1.5	.....	2	441	11,000 Est.	O.	O.
2.5	.....	3	397	8,000	O.	O.
4.0	1 × 0.15‡	4	442	5,600	A. O.	O.
6.5	1 × 0.15‡	5	453	5,600	A. O.	O.
9.5	2 × 0.15‡	6	396	1,300	A. O.	O.
16.0	2 × 0.15‡	7	415	890	2,200	3,000 Est.
22.0	3 × 0.15‡	8	420	894	5,000	2,000 Est.
28.0	5 × 0.15‡	9	548	692	2,300	A. O.
42.0	3 × 0.15‡	10	416	701	542	27
49.5	1 × 0.15‡	11§	382	432	1,200	38
49.5	1 × 4.15§	12¶	411	602	1,000	634
53.5	5 × 0.15‡	13	435	614	1,000	24
66.6	3 × 0.15‡	14#	402	478	654	140

\* Inoculation: 0.001 c.c. of four-hour broth culture of pneumococcus, Group II.

† Intramuscularly.

‡ Orally.

§ Immediately before 2 c.c. of extract of antipneumococcus serum, Group II, intravenously.

¶ Immediately after the above dose of extract, and immediately before 10 c.c. of the same extract subcutaneously.

# Nine hours after a second dose of 10 c.c. of extract of antipneumococcus serum, Group II, subcutaneously.

Table 19 shows that in this case (Case 15, Hosp. No. 2604), in which the quantity of drug administered was 1.5 gm. per twenty-four hours (of which the first dose of 0.45 gm. was given intramuscularly), equivalent to 0.270 gm. per kilogram of body weight, the bactericidal action did not appear in the serum until somewhere between twenty-eight and forty-two hours after the initial dose. Here again the temporary inhibition following the intramuscular injection was by no means marked.

From the three foregoing cases it would appear that the intramuscular route is less satisfactory than the oral route.

## ABSORPTION AND ELIMINATION

Some knowledge of the rates of absorption and of elimination or destruction of optochin is desirable from a therapeutic standpoint, in order that the intervals between individual doses be not so long as to permit the bactericidal action to disappear from the circulating blood. Methods for the quantitative determination of the small amounts of optochin involved are not available, so that for information needed on this point recourse must be had to a biologic method, such as that employed in this investigation.

We have seen that optochin is rapidly absorbed from the gastrointestinal tract, so that within less than three hours after 0.5 gm. has been taken by mouth it is possible to detect the specific inhibitory action in the patient's blood. Table 3 shows that at the end of six hours after such a dose the inhibitory action was greater than at the end of eight hours, which indicates that the drug at this latter time was being eliminated or destroyed. When, now, a second dose of 0.5 gm. was given by mouth, tests conducted at the end of eight hours after this dose showed a greater inhibitory or bactericidal action than those at the end of the first eight hours. Similar results are seen in Table 4. These facts indicate that, under such conditions of dosage, the rate of absorption of the drug is faster than that of elimination or destruction, as a result of which a positive balance is established and a certain amount of the drug retained in the body. When numerous comparatively small doses of the drug are given in twenty-four hours (for example, 0.15 gm. every two and one-half hours) a steady increase is observed in the inhibitory power of the blood until bactericidal action is manifest, as shown by Tables 5 and 6. This again indicates that part of the drug is being retained in the body. Such retention lasts only so long as the drug continues to be supplied in sufficient quantity, for when it is discontinued the blood soon loses its bactericidal power. Wright<sup>4</sup> has shown that the drug is eliminated in the urine. It is possible that it may be in part destroyed in the body. Elimination probably proceeds at a fairly rapid rate, as indicated above, hence it is important, in the treatment of patients, that the intervals between the individual doses should not be too great. When the patient is receiving numerous doses of 0.15 gm. each and has in his blood a bactericidal action, it may happen that, if four hours or more are allowed to intervene between the individual doses, the bactericidal action may disappear. The following case illustrates this point.

CASE 16 (No. 2582).—S. S., tobacconist, aged 48, weight 45.3 kg.

*Past History.*—Unimportant.

*Present Illness.*—Chill, pain in chest, cough and bloody sputum two days before admission.

Status on Admission: temperature 102.2 F., pulse 90; respirations 24. Signs



indicating beginning involvement of right upper lobe; blood culture sterile; white blood count 13,400; sputum tenacious, rusty, containing *Pneumococcus mucosus* (Group III).

Course: Optochin was started on the third day of the disease; all doses were given by mouth. The patient was given 0.4 gm. as initial dose and six doses 0.15 gm. each in first 24 hours, ten doses 0.15 gm. each in second and third twenty-four hours; the drug was then omitted for thirteen and one-half hours and then resumed as follows: in periods of twenty-four hours each, 0.45 gm. + 8 x 0.15; 7 x 0.15; 6 x 0.15; 5 x 0.15, in sixteen hours making a total of 8.65 gm. in all. Two days after admission and thirty-six hours after optochin was started, signs of consolidation of right upper lobe became well marked. The general condition of the patient grew worse for several days after optochin was started, respirations reaching 62 per minute, and he appeared extremely ill. The pneumonic process did not spread to any other lobe, however, and on the ninth day the temperature began to fall, reaching 99.8 F. on the eleventh day, when the optochin was discontinued. Physical signs on this day indicated that resolution

TABLE 20.—BACTERICIDAL TEST OF SERUM OF CASE 16 (No. 2582)

Time in Hours after Initial Dose of Optochin	Doses of Optochin in Grams Following Corresponding Test Bleeding	Specimen of Serum Tested	Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*			
			Immedi-ately	After 3 Hours Inc.	After 7½ Hours Inc.	After 21¼ Hours Inc.
0.0	1 x 0.4	1	567	6,346	C.	C.
1.75	1 x 0.15	2	515	277	242	16
4.75	1 x 0.15	3	569	263	244	13
7.75	1 x 0.15	4	557	383	221	35
12.0	1 x 0.15	5	603	304	209	88
15.75	1 x 0.15	6	490	338	386	2,800
19.75	1 x 0.15	7	588	449	723	5,000
23.75	4 x 0.15	8	476	488	953	A. C.
35.75	6 x 0.15	9	467	381	328	39
47.75	1 x 0.15	10	500	327	75	1
50.75	3 x 0.15	11	562	288	74	0
59.75	4 x 0.15	12	536	235	60	1
67.75†	2 x 0.15	13	486	261	65	0
	1 x 0.45 8 x 0.15					
109.0	6 x 0.15	14	264	97	7	0
132.25	6 x 0.15	15	335	95	16	0
156.25	5 x 0.15	16	272	85	11	0
177.0	.....	17‡	354	132	29	2
184.0	.....	18§	269	181	291	33
206.0	.....	19¶	299	1,000	C.	C.

\* Inoculation: 0.00001 c.c. of a nineteen-hour broth culture of pneumococcus, Group II.

† Optochin stopped for thirteen and one-half hours, then resumed.

‡ Three hours after last dose.

§ Ten hours after last dose.

¶ Thirty-two hours after last dose.

was in progress. The patient became slightly deaf while receiving the drug, but this condition disappeared when the drug was stopped.

Amount of optochin per kilo of body weight per twenty-four hours; first twenty-four hours = 0.0286 gm.; second and third twenty-four hours = 0.033 gm.

It will be seen from a study of Table 20 that this patient (Case 16, Hosp. No. 2582) received an initial dose of 0.4 gm. and that within two hours his serum had acquired bactericidal power. The second dose, 0.15 gm., was given two hours after the first, and there then followed two doses of 0.15 gm. at intervals of three hours, during which time the serum was constant in its bactericidal activity. During the remaining three fourths of the first twenty-four hours the doses of 0.15 gm. were given at intervals of from three and three-fourths hours to four and one-half hours, and it is significant that between the twelfth hour and the twenty-fourth hour bactericidal action disappeared and was replaced by only slight inhibition (Specimens 6, 7 and 8). From the twenty-fourth to the thirty-sixth hour the drug was given in doses of 0.15 gm. every two or three hours, and the specimen of serum obtained about the thirty-sixth hour of treatment showed definite bactericidal activity. In this patient it would seem that, early in the course of treatment, the rates of absorption and elimination were comparatively rapid, and, also, that a period of four hours constituted too long an interval between individual doses of 0.15 gm. each.

Case 8 (Hosp. No. 2566), Table 10, illustrates the same principle. Hence, in administering frequent small doses of optochin to patients, if it be desired to establish and maintain a constant bactericidal action in the serum, too great an interval of time must not elapse between single doses.

In Case 16 (Hosp. No. 2582), Table 20, the protocol shows that after the patient had received optochin for more than seven days a specimen of serum obtained ten hours after the last dose still showed bactericidal action. This would suggest that when adequate administration of the drug lasts over a comparatively long time, considerable accumulation of the drug may take place, so that bactericidal action in the serum is maintained for a longer time than would be expected after a shorter course of administration according to the same dosage. A similar observation has been made in several of our cases. The possibility occurs, therefore, of diminishing the dosage after several days' administration of the drug, and still maintaining the bactericidal action in the serum.

In Case 6 (Hosp. No. 2673, Table 8), however, in the last twenty-four hours of treatment, that is on the fifth to sixth day, the amount of the drug given per kilogram of body weight was reduced to 0.016 gm., and the bactericidal action disappeared from the serum four hours after the last dose.

From a therapeutic standpoint it is of importance to know whether or not optochin can pass from the blood through the walls of the capillaries of the lung into the alveolar spaces. No data are available on this point. That the drug is capable of passing through the capillary walls into the serous sacs, however, is indicated by observations made on one case, Case 2 (Hosp. No. 2705, Table 4). This patient received optochin over a period of seven days and in such amounts that a decided bactericidal action became manifest in the serum within twenty-four hours after the initial dose, and remained constant throughout the course of treatment. In spite of this fact the disease process was not permanently arrested and the patient died. Immediately after death, 40 c.c. of turbid yellow fluid were aspirated from the pericardial cavity. This fluid clotted shortly after removal and was found by cultural tests to be sterile. The serum was removed from the clot and tested in the usual manner for bactericidal action on the pneumococcus. As controls, normal human serum from another patient, ascitic fluid from a patient with cirrhosis of the liver and blood serum from the patient herself obtained at the height of administration of the drug, were used. The patient's own blood serum before optochin was administered had already been shown to possess no bactericidal action for the pneumococcus. The results of the experiment are given in Table 21.

TABLE 21.—BACTERICIDAL TEST OF PERICARDIAL FLUID OBTAINED FROM CASE 2 (No. 2705) (TABLE 4)

Fluid	Number of Colonies of Pneumococci per 0.5 C.c. When Plated*			
	Immediately	After 1½ Hours Inc.	After 5 Hours Inc.	After 21 Hours Inc.
Normal blood serum .....	138	780	4,000 Est.	O.
Ascitic fluid .....	157	638	1,500 Est.	C.
Blood serum, Case 2 (Hosp. No. 2705) (optochin for six days by mouth).....	104	153	23	0
Pericardial fluid, Case 2 (Hosp. No. 2705), immediately after death.....	132	104	33	0

\* Inoculation: 0.001 c.c. of four-hour broth culture of pneumococcus, Group II.

Table 21 shows that the pericardial fluid obtained from the patient, Case 2 (Hosp. No. 2705) immediately after the death of the patient, and the blood serum of the same patient obtained about twenty-four hours before death and after optochin had been given by mouth for six days, both showed marked bactericidal activity for the pneumococcus; whereas, normal blood serum and ascitic fluid obtained from a case of cirrhosis of the liver showed no such bactericidal activity. It would seem justifiable to conclude from these observations that opto-



chin is capable of passing from the blood, through the capillary wall, into the pericardial cavity. This fact is of importance as regards the permeability of the capillaries for optochin and also because of the bearing it has on the possible influence of the drug on the occurrence of empyema or pericarditis in cases of lobar pneumonia.<sup>26</sup>

#### "FASTNESS"

As stated previously, pneumococci, when subjected to the action of concentrations of optochin not sufficiently great to destroy them, may react against the drug in such a manner that they attain a condition in which they are uninfluenced by concentrations of the drug formerly great enough to bring about their death. This state of resistance or "fastness" may be acquired within the animal body (mice) as well as in the test tube. In lobar pneumonia the conditions may be not unfavorable for the acquisition of this characteristic by the pneumococci. Thus, in the consolidated portions of the lung the organisms may be protected mechanically from the action of the full strength of the circulating drug, by reason of a possible relative impermeability of the exudate for optochin, or other factors.

In one of our cases in which the patient was treated consistently and according to what would seem to be the optimum method of dosage, it was possible to recover from the blood immediately after death a strain of pneumococcus which was definitely "fast" toward the drug; whereas the strain recovered from the blood before optochin treatment was begun did not exhibit this characteristic. The details of this case follow.

CASE 17 (No. 2638).—C. G., pedler, aged 55, weight 42.8 kg.

*Past History.*—Unimportant.

*Present Illness.*—Cough for several days; became worse two days before admission, when patient began to have pain in chest.

*Status on Admission:* temperature, 103.8 F.; pulse, 106; respirations, 40; involvement of part of right lower lobe; roentgenogram shows shadow in this area; white blood count, 18,000; sputum tenacious, slightly blood streaked, containing pneumococcus, Group IV. Blood culture showed pneumococcus, Group IV.

*Course:* Optochin started on the fourth day of disease; all doses given by mouth. During the first twenty-four hours the patient received a first dose of 0.5 gm., a second dose of 0.15 gm. and seven doses of 0.1 gm. each. In the second, third and fourth periods of twenty-four hours each he received twelve doses of 0.1 gm.; during the fifth period, seven doses of 0.1 gm. each and three of 0.15 gm. each; during the sixth period, nine doses of 0.15 gm. each. On the seventh day of the disease (third day of treatment) the involved area began to increase in extent and on the ninth day the entire right lower lobe was consolidated. At this time the patient was quite ill, but shortly afterward he seemed to improve somewhat, and on the tenth day of the disease the lowest temperature was 99.4 F.; the highest 101.6 F. On the eleventh day (seventh day of treatment)

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26. Since the foregoing was written we have been able to demonstrate pneumococidal action in the pericardial fluids obtained from two other patients who had received optochin hydrochlorid by mouth for several days.

the temperature was 99.2 F. (rectal). The drug was discontinued. On the following day the temperature rose again and the patient's condition grew rapidly worse. At this time physical signs indicated involvement of the left lower lobe. Optochin was immediately resumed (after an intermission of seven and one-half hours) as follows: 0.5 gm., followed by two doses of 0.1 gm. each. The patient died rather suddenly that same day (twelfth day of disease). The physical signs in the right lung indicated that at that time the pneumonic process was beginning to resolve. A culture of the heart's blood taken immediately after death showed pneumococcus, Group IV, and a strain of pneumococcus belonging to the same immunologic group was recovered from the left lower lobe at the same time. No pneumococcus was obtained by aspiration of the right lower lobe.

Total amount of optochin given, 8.75 gm. No toxic symptoms referable to the drug were observed. Amount of optochin per kilo per twenty-four hours, 0.031 gm. first twenty-four hours, 0.028 gm. second, third and fourth periods.

TABLE 22.—BACTERICIDAL TEST OF SERUM IN CASE 17 (No. 2638)

Time in Hours after Initial Dose of Optochin	Doses of Optochin in Grams Following Corresponding Test Bleeding	Specimen of Serum Tested	* Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*			
			Immediately	After 1½ Hours Inc.	After 5½ Hours Inc.	After 22½ Hours Inc.
0.0	1 × 0.5	1	305	7,100	C.	C.
3.0	1 × 0.15	2	331	373	15,000 Est.	C.
6.0	1 × 0.1	3	337	308	395	3,000 App.
9.0	1 × 0.1	4	283	281	395	484
11.0	1 × 0.1	5	334	321	337	89
13.0	2 × 0.1	6	262	295	285	275
16.5	1 × 0.1	7	330	326	...	10
21.0	2 × 0.1	8	350	367	...	11
25.5	2 × 0.1	9	312	319	303	7
29.5	2 × 0.1	10	333	319	289	3
33.75	5 × 0.1	11	360	322	335	4
43.5	2 × 0.1	12	314	358	175	3
47.5	3 × 0.1	13	324	331	208	1
53.5	9 × 0.1	14	263	285	231	1
71.0	12 × 0.1	15	326	338	137	35
97.0	7 × 0.1 4 × 0.15	16	334	294	206	2
123.0	8 × 0.15	17	373	337	189	3
147.5	4 × 0.15 1 × 0.5 2 × 0.1	18	343	323	131	1

\* Inoculation: 0.001 c.c. of four-hour broth culture of pneumococcus, Group II.

In Case 17 (Hosp. No. 2638) the amount of drug given per kilogram of body weight for the first twenty-four hours was 0.031 gm., and later 0.028 gm.; bactericidal action was manifest eleven hours after the initial dose, and remained constant thereafter throughout the course of administration; in spite of this fact, however, the pneumonic

process spread to previously uninvolved portions of the lung and the patient died. The blood culture on admission showed the presence of pneumococcus, Group IV. Unfortunately, no other blood cultures were made during life, but a culture from the heart's blood, made immediately after death, yielded an organism belonging to the same immunologic group as did a culture from the left lower lobe (the advancing lesion) made at the same time. A culture made from the lesion in the right lung, just after death, was sterile. In all, four strains belonging to the same immunologic group were obtained from this patient (including those isolated from the sputum and blood on admission). All four strains were tested at the same time with regard to the presence or absence of "fastness" toward optochin. This was done (1) by observing the growth of the various strains in varying concentrations of the drug in nutritive bouillon, and (2) by comparing their behavior in normal serum with that in serum obtained from the patient at the height of the administration of optochin, when his serum possessed a strong pneumococcal action (Experiment 2).

*Experiment 2.*—Eighteen-hour broth cultures of all four strains were used. Dilutions of optochin hydrochlorid in broth, varying from 1: 10,000 to 1: 5,000,000, were freshly made up, tested for sterility, and into tubes containing 5 c.c. of each of these dilutions 0.05 c.c. of each of the above-mentioned cultures was inoculated. The tubes were then incubated at 37 C. and readings made at the end of twenty-four hours and forty hours. Specimens of the eighteen-hour cultures used for incubation were diluted and the dilutions plated in order to be sure that the inoculations were comparable. That they were comparable is shown by Table 23. For convenience the strains have been designated as follows:

Strain A, isolated from the sputum on admission.

Strain B, obtained from the blood on admission.

Strain C, obtained from the left lower lobe immediately after death (spreading lesion).

Strain D, obtained from heart's blood after death.

TABLE 23.—NUMBER OF COLONIES OF PNEUMOCOCCI PRESENT IN 0.00001 C.C. OF EIGHTEEN-HOUR BROTH CULTURES OF STRAINS A, B, C AND D

Dilution, C.c.	Strain A	Strain B	Strain C	Strain D
0.00001	944	2,168	2,228	1,120

Table 23 shows that more or less comparable numbers of bacteria were present in the cultures, of each of which 0.05 c.c. was used for inoculation.

The amount of growth in the tubes (Table 24) was judged macroscopically, and in addition smears from tubes 1 to 5, inclusive, Strain D and from Tube 1, Strain B, showed gram-positive diplococci after forty hours' incubation. From the first three tubes of each strain which showed no growth after forty hours' incubation, 0.5 c.c. was removed and plated in about 20 c.c. of 1 per cent. glucose agar. These plates after forty-eight hours' incubation showed no colonies.



TABLE 24.—GROWTH OF EIGHTEEN-HOUR BOUILLON CULTURES OF STRAINS A, B, C AND D IN VARYING DILUTIONS OF OPTOCHIN IN BROTH AT 37 C.

Tubes	Dilution of Optochin in Broth	Strain A		Strain B		Strain C		Strain D	
		22 Hrs.	40 Hrs.	22 Hrs.	40 Hrs.	22 Hrs.	40 Hrs.	22 Hrs.	40 Hrs.
1	1:5,000,000	0	0	0	Heavy	0	0	Heavy	Heavy
2	1:1,000,000	0	0	0	0	0	0	Heavy	Heavy
3	1:750,000	0	0	0	0	0	0	Heavy	Heavy
4	1:500,000	0	0	0	0	0	0	Heavy	Heavy
5	1:250,000	0	0	0	0	0	0	Heavy	Heavy
6	1:100,000	0	0	0	0	0	0	0	0
7	1:50,000	0	0	0	0	0	0	0	0
8	1:10,000	0	0	0	0	0	0	0	0
Control broth without optochin.....		Heavy		Heavy		Heavy		Heavy	

TABLE 25.—COMPARISON OF GROWTH OF STRAINS A, B, C AND D IN NORMAL HUMAN SERUM AND IN BACTERICIDAL SERUM OBTAINED FROM CASE 17 (No. 2638), DESIGNATED SERUM G

Strain	Serum	Number of Colonies of Pneumococci per 0.5 C.c. Serum When Plated*			
		Immediately	After 2½ Hours Inc.	After 7½ Hours Inc.	After 21 Hours Inc.
A	Normal	207	822	A. C.	C.
A	G	232	58	7	0
B	Normal	218	796	C.	C.
B	G	924	375	5	0
C	Normal	434	10,000 Est.	C.	C.
C	G	398	43	4	1
D	Normal	730	A. C.	C.	C.
D	G	737	10,000 Est.	C.	C.
Group II	Normal	229	400	C.	C.
Group II	G	263	32	0	0

\* Inoculation: 0.00001 to 0.000001 c.c. of eighteen-hour broth cultures.

From Table 24 it is seen that Strains A and B, isolated from the sputum and blood, respectively, on admission and before the patient had received any optochin, and Strain C, isolated from the left lung after death, grew readily in ordinary broth, but were killed in very high dilutions of the drug in broth (1:1,000,000 to 1:5,000,000); on the contrary, Strain D, isolated from the heart's blood immediately after death, grew freely in much greater concentrations of the drug and was only killed in a concentration of 1:100,000 or greater. Furthermore, as shown by Table 25, when incubated in serum obtained from the

patient himself, which showed a definite pneumococcal action, strains A, B and C and a strain belonging to Group II were readily killed, whereas Strain D was not killed, but grew almost as readily in this serum as in normal human serum.

The facts brought out in these experiments are clear: A strain of pneumococcus isolated from the heart's blood immediately after death was "fast" toward optochin; whereas, strains isolated from the sputum and blood before the administration of optochin and from the lesion in the left lung after death, did not show this condition of fastness. This fastness or resistance to optochin of Strain D was acquired at the most within eight days after the drug treatment was started, and all tests made during this time showed a decided bactericidal action in the patient's blood serum. The actual mode of development of the fastness and the conditions favoring its production are not clear.

In the last twenty-four hours of the patient's life the drug was omitted for a space of seven and one-half hours, after which time it was resumed and a comparatively large dose, 0.5 gm., given at once, followed by two doses of 0.1 gm. It seems unlikely that during this intermission of the drug treatment the concentration of optochin in the blood fell to such a point that fastness was acquired within this time; as we saw in Case 16 (Hosp. No. 2582) (preceding section) that when optochin is given over several days there may be evidence of retention or accumulation of the drug, inasmuch as the serum showed a bactericidal action for as long as ten hours after the last dose.

The possibility exists that a certain degree of fastness was acquired by some of the organisms in the peripheral part of the consolidated area, and that this condition was sufficient to permit them, during the time that the treatment was omitted, to invade the blood stream where they could, during the intermission, and later, conceivably develop a still greater degree of resistance to the drug. It is probable, however, that the fatal outcome of the case is not to be attributed necessarily to the fact that the drug was discontinued for a brief interval. Nevertheless, the fact that the infecting micro-organism was able to become so resistant to the concentration of optochin present in the blood serum during treatment that invasion of the blood stream was not prevented, was probably an important factor in this case in preventing a successful outcome from optochin treatment.

#### TOXIC EFFECTS

Optochin in too large amounts is toxic for laboratory animals, and in the clinical use of the drug certain toxic symptoms have been observed. So far as known, only one human death has been, in part, attributed to the use of the drug, and in that case it was given intravenously;<sup>10</sup> death was in that case considered to be due to heart failure,

in part caused by the use of optochin; even in this case it is not entirely clear that heart failure was occasioned by the optochin. The toxic symptoms noticed have generally been transient and have usually disappeared when the drug was discontinued. The principal ones that occur are referable to the eye and the ear. In some cases vomiting has been reported; this may be due in some instances to the bitter taste of the drug, which rivals quinin in that respect. Vomiting, however, is rather infrequent, and one disadvantage connected with it is the possibility that the drug may be ejected. In only one of our cases was vomiting frequent enough to interfere with the administration of the drug, and in that case it is doubtful if the optochin was the cause.

The symptoms referable to the ear comprise ringing and roaring noises and partial deafness. In six of our cases tinnitus and partial deafness were associated, and in one case there was tinnitus alone, without deafness. In every case these symptoms were transient, disappearing shortly after the drug was discontinued. Indeed, in some instances these symptoms disappeared or became less evident while the drug was still being given in full amounts.

The symptoms referable to the eyes are more serious. It is difficult to determine the percentage of recorded instances of eye trouble due to the use of the drug; a review of the literature would seem to show that about 4.57 per cent. of all reported treated patients have had transient amblyopia or amaurosis. In one recorded case there has been permanent, almost complete, blindness. In this case, reported by Oliver, the patient received 5 grains (0.3 gm.) every three hours, which would mean 2.4 gm. per twenty-four hours (total amount 7.7 gm.), a dose probably greater than that usually given. In all other cases of amblyopia and amaurosis reported in the literature, the visual disturbances apparently disappeared after the drug had been discontinued. With the impairment of vision the pupils may be widened, may not react to light, and ophthalmoscopic examination may show tortuosity of the retinal vessels, and generally shows narrowing of the arteries. Whether these changes in the pupils and vessels precede the impairment of vision for any considerable time cannot be said at present; they did not appear in any of our cases with the exception of the one that had already developed amaurosis, although all of the patients who received the drug had daily ophthalmoscopic examinations. It is interesting to note that the ophthalmoscopic picture in optochin amblyopia is said to resemble closely that of quinin amblyopia.<sup>27</sup>

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27. Since the foregoing was written L. Loránt (*Deutsch med. Wchnschr.*, 1916, **42**, 1355) has described a case of total amaurosis in a woman who apparently received 2.4 gm. of optochin hydrochlorid per twenty-four hours, in doses of 0.2 gm. Vision was only slightly restored six months afterward. Recently, also, we have observed transient amblyopia, lasting one to six hours, in three patients who received optochin hydrochlorid by mouth in amounts sufficient to produce pneumococidal action in the blood stream. In our cases administration of the drug was discontinued on the first appearance of eye symptoms.



There is some ground for believing that the visual disturbances may depend to a certain extent on idiosyncrasy. That a condition of susceptibility to the drug may be an important factor is indicated by the fact that some patients develop amblyopia after they have had only 2 gm. or less of optochin, whereas others do not acquire it even after they have received as much as from 10 to 16 gm. Study of the literature seems to indicate, however, that the more the daily dosage is subdivided, the less frequent are the visual disturbances. If subsequent observation bears out this point, an additional reason is supplied for not giving the drug in three large doses in twenty-four hours, which method, as shown previously, probably gives rise to considerable fluctuations in the concentration of optochin in the circulating blood. It is possible, also, that the maintenance of the body-weight relationship may help to diminish the frequency of occurrence of this complication by preventing overdosage. Daily examination of the eyegrounds and the pupils may give warning of impending serious symptoms and thus indicate the discontinuance of the drug, or reduction of dosage.

One of the patients included in the present study complained of photophobia during the course of administration of the drug, but on close questioning the patient admitted that this symptom was present before admission to the hospital; the optochin was not discontinued and no impairment of vision resulted. Another patient (Case 18, Hosp. No. 2573) developed an acute retinitis with temporary blindness after receiving four doses of 0.5 gm. each at intervals of eight hours, and deserves to be recorded in detail. For the ophthalmoscopic notes we are indebted to Dr. Otto Schirmer of New York, who followed the case closely and with interest.

CASE 18 (No. 2573).—Aged 16, weight 52.6 kg.

*Past History.*—Unimportant.

*Present Illness.*—Cough and pain in the chest two days before admission.

Status on Admission: temperature, 103.4 F.; pulse, 120; respirations, 40; involvement of right lower lobe; white blood count, 18,000; blood culture sterile; sputum tenacious, slightly rusty, containing pneumococcus, Group II.

Course: Optochin started on fourth day of disease. All doses were given by mouth. Four doses of 0.5 gm. each were given at eight-hour intervals. After receiving 2 gm. the patient suddenly complained of inability to see. The pupils were found to be dilated and did not react to light or on accommodation. The patient could not distinguish a bright light when flashed directly before her eyes. Ophthalmoscopic examination showed reddish disks, with blurred outlines, gray retinae, tortuous arteries and dilated veins. Diagnosis: acute toxic retinitis (Dr. Schirmer). The administration of optochin was discontinued at this point. The temperature fell by crisis on the eighth day. After six days of total blindness the patient could distinguish light from darkness. Her vision gradually returned, the disks lost their reddish color, the retinae became less hazy, and the arteries became contracted and lost their tortuosity. Three months after the temporary blindness, central vision was 15/20 with each eye; visual fields were contracted to about 10 to 15 degrees and the arteries remained narrow.

Seven months later the visual fields had appreciably increased in extent, and

vision was 15/15 with each eye. The arteries were still contracted; the disks were slightly pale. The patient is working steadily in a factory, and the only abnormal thing she notices is fatigue of the eyes after reading.

The total amount of optochin given was 2 gm. Amount of optochin per kilo of body weight, per twenty-four hours = 0.0285 gm.

The occurrence of amblyopia in 4.57 per cent. of all published cases in which the patients have been treated with optochin constitutes a distinct drawback in the use of the drug. The danger of permanent impairment of vision as a result of the use of optochin should always be borne in mind. Patients undergoing treatment with the drug should be carefully watched and questioned with this possibility in view.

#### INFLUENCE ON THE CLINICAL COURSE OF THE DISEASE

In attempting to determine the effect of optochin treatment on the course of the disease in the patients reported in this series, the cases will be analyzed from the following points of view: (1) the occurrence, during optochin treatment, of involvement of a hitherto uninfected lobe of the lung, designated as "spread" for brevity's sake; (2) the occurrence of pneumococcal septicemia before or during treatment, or after treatment was discontinued; (3) the duration of the disease, and (4) the mortality rate.

1. *The Occurrence of "Spreads."*—The involvement of a hitherto unaffected lobe during the course of lobar pneumonia is an unfavorable sign. It is an indication that the resistive powers of the individual have not succeeded in arresting the progress of the disease, and it constitutes an additional increment to the burden which the patient is compelled to bear as a result of the infection. In view of these considerations it would seem that the prevention of these so-called "spreads" during the course of specific treatment of lobar pneumonia may be taken as some measure of the ability of the treatment to arrest the progress of the disease.

In seven of the thirty-two patients treated with optochin the pneumonic process spread to an uninvolved lobe during the course of treatment. In judging whether or not such a spread had occurred, we have been guided by the physical signs for the most part, aided, in some instances, by Roentgen-ray or necropsy examinations. It is recognized that involvement of a lobe may take place many hours before definite indication of such is given by the physical signs; this fact has been taken into consideration when deciding whether or not a spread had occurred during treatment. Thus, if the physical signs indicating involvement appeared within less than twenty-four hours after the drug was first given, it was considered that the involvement had actually taken place before treatment had been begun. Some such definite time limit is necessary if the observations are to have any value.



Although seven cases showed such a spread during treatment, an analysis shows that in one instance (Hosp. No. 2466) the patient vomited frequently during the administration of the drug, so that it is impossible to say whether or not she received a sufficient amount of the drug. (No bactericidal tests of the serum were made in this case.) In another case (Case 9, Hosp. No. 2581) the amount of drug given per kilogram of body weight per twenty-four hours was 0.0187 gm., and the bactericidal tests showed that this amount was insufficient to produce bactericidal action at any time in the serum of the patient; consequently, in this instance, it could scarcely be expected that the disease would be arrested by the use of the drug. As a matter of fact, the condition of this patient grew steadily worse for several days while receiving the drug. Another patient (Hosp. No. 2499), weighing 80.4 kg., who received 0.0186 gm. of optochin per kilogram of body weight per twenty-four hours showed evidences of a spread during treatment. Unfortunately no bactericidal tests were carried out in this case, but, from the foregoing studies we should expect that the amount of optochin given was insufficient to produce bactericidal action in the serum. Still another patient (Case 14, Hosp. No. 2593, p. 650), who received an amount insufficient to produce bactericidal action in the serum, as shown by tests, gave definite indications that a spread took place during treatment. Of the seven patients who exhibited a spread of the pneumonic process during administration of optochin, probably three received an amount of the drug which sufficed to produce in the serum a decided bactericidal action on pneumococci.

2. *Pneumococcal Septicemia*.—It would seem that if, under optochin therapy, the blood serum acquires the property of killing pneumococci in the test tube, the presence of pneumococcal activity in the blood stream should be able to prevent the occurrence of a pneumococcal septicemia or to destroy the micro-organisms already present in the blood stream. Leschke<sup>28</sup> and others saw favorable results from optochin treatment in pneumococcal septicemia.

In six of the cases which are included in this study, positive blood cultures<sup>29</sup> were obtained before the administration of optochin was begun. Of these six, two (Cases 1 and 6, Hosp. Nos. 2600 and 2673) gave negative blood cultures twenty-four hours after the initial dose of optochin, and in each case tests showed the presence of bactericidal action in the patient's serum at that time. Of the remaining four, one (Case 12, Hosp. No. 2711) still showed a positive blood culture twenty-four hours after the initial dose of optochin and at a time when com-

28. Leschke, E.: Deutsch. med. Wchnschr., 1915, **41**, 1359; München. med. Wchnschr., 1914, **61**, 2433; Berl. klin. Wchnschr., 1915, **61**, 634.

29. Ten cubic centimeters of blood were incubated in 150 c.c. of broth as a routine.



plete inhibition, but not bactericidal action, was manifest in the blood serum; one (Hosp. No. 2466) showed a progressive increase in the number of pneumococci in the blood, but vomited frequently during the course of administration of the drug, so that undoubtedly considerable optochin was lost (no bactericidal tests were made); one (Hosp. No. 2508, alcoholic, delirium tremens) died sixteen hours after the initial dose of the drug and before a second blood culture could be made; and one (Case 17, Hosp. No. 2638) showed in the blood immediately after death pneumococci which were "fast" to the concentration of the drug present in the blood stream of the patient during treatment, a concentration which sufficed to kill the strain present in the blood and sputum before treatment was begun. Thus in two of the six cases there is some ground for believing that the optochin may have been instrumental in ridding the circulating blood of living pneumococci.

One patient (Case 14, Hosp. No. 2593) gave a negative blood culture immediately before the optochin was commenced, but shortly before death there were four colonies of *Pneumococcus mucosus* per 1.0 c.c. of blood. During the last thirty hours of life this patient received no optochin, owing to inability to swallow the drug.

These observations do not afford much information concerning the value of optochin treatment on the course of pneumococcal septicemia in lobar pneumonia, except in the one instance in which a strain of pneumococcus was shown to acquire a condition of fastness within the body of the patient.

It is possible that the persistence or occurrence of a pneumococcal septicemia after the patient has received optochin for a period of twenty-four hours does not necessarily indicate that the drug is not able to cope with the septicemia, for in some instances more than twenty-four hours may be necessary for the drug to destroy all the organisms in the circulating blood, as indicated by the bactericidal tests; moreover, the presence of a few pneumococci in the blood stream at any one moment does not necessarily mean that they have been there continuously for a considerable period of time, or that they are actually multiplying in the blood, for it is possible that they may be continually gaining access to the circulating blood from the infected focus, where they may be protected from the action of the optochin in full strength.

3. *Duration of the Disease.*—The determination of the duration of an attack of lobar pneumonia, when crisis does not occur, is not easy; in analyzing the cases from this standpoint it has been necessary to set arbitrary standards as criteria of the termination of the active process. Where the disease did not terminate in a crisis which was unmistakable, it has been considered that the acute process had ended when the rectal temperature reached 100 F., and the patient was obvi-

ously in a satisfactory condition. This method has, of course, disadvantages, but it will serve as a basis for discussion.

The average duration of the disease in twenty-three of the twenty-four cases in which there was recovery was 8.1 days.<sup>30</sup> In twelve of these twenty-three cases optochin treatment was started on or before the third day of the disease and the average duration of the disease in these cases was 7.4 days; the average duration of the disease in the eleven cases in which the optochin treatment was begun after the third day of the disease was 8.9 days. Thus, any considerable shortening of the duration of the disease was not apparent in our cases.

4. *Mortality Rate*.—As a result of the work of Cole<sup>31</sup> and his associates, considerable light has been thrown on the immunologic relationships of the pneumococci found in cases of acute lobar pneumonia. On the basis of certain immunity reactions it has been found possible to divide the pneumococci found in lobar pneumonia into four general groups. In three of these (Groups I, II and III) the members of each group show certain complete cross immunologic reactions among themselves, but not with members of any other group. Group III consists of organisms which, on the basis of cultural and morphologic characteristics, are identical with the type known as *Pneumococcus mucosus*. The individual members of Group IV, thus far, have not been found to show cross immune reactions with one another, or with members of Groups I, II or III.

It has been found that the percentage of cases of lobar pneumonia due to pneumococci of one or other of these groups may vary somewhat from year to year and in different clinics; moreover, the pneumococci belonging to these various groups show quite marked differences, among other things, in their virulence both for human beings and for experimental animals, so that considerable variations are encountered in the mortality rate of cases of lobar pneumonia, depending on the particular group to which the infecting pneumococcus belongs.<sup>32</sup> In view of these facts, a consideration of the mortality rate in any series of cases of lobar pneumonia, treated according to a particular method, is not of full value unless the cases be studied from the standpoint of the groups to which the infecting pneumococci belong. This consideration will be more clearly brought out by a study of Tables 26 and 27 (Cole<sup>33</sup>), which show the average incidence of the various

30. The one patient that received large doses of antipneumococcus serum, Group I, is excluded.

31. Cole, R.: THE ARCHIVES INT. MED., 1914, **14**, 56. Dochez, A. R., and Gillespie, L. J.: Jour. Am. Med. Assn., 1913, **61**, 727. Avery, O. T.: Jour. Exper. Med., 1915, **22**, 804.

32. Cole, R.: New York Med. Jour., 1915, 1 and 58. Mathers, G.: Jour. Infect. Dis., 1915, **17**, 514. Cole, R.: Trans. Cong. Am. Phys. and Surg., 1916, **10**, 138.

33. Cole, R.: Tr. Cong. Am. Phys. and Surg., 1916, **10**, 138.

serologic groups of pneumococci in lobar pneumonia and the mortality rate in each group. The figures have been collected from three hospitals and together they constitute a representative group of cases of this infection.

TABLE 26.—INCIDENCE OF SEROLOGIC GROUPS OF PNEUMOCOCCI IN CASES OF LOBAR PNEUMONIA

Institution	Number of Cases				Per Cent.			
	Group I	Group II	Group III	Group IV	Group I	Group II	Group III	Group IV
Hospital of Rockefeller Institute.....	113	104	37	74	34.0	31.0	11.0	22.0
Pennsylvania Hospital.....	32	26	3	34	33.0	27.0	3.0	36.0
Cook County Hospital <sup>34</sup> .....	50	25	5	31	45.0	22.5	4.5	28.0
	195	155	45	139	36.0	29.0	8.4	26.0
	Total cases, 534							

TABLE 27.—VARIATIONS IN MORTALITY OF CASES OF LOBAR PNEUMONIA NOT SPECIFICALLY TREATED DEPENDING ON THE SEROLOGIC GROUP OF INFECTING PNEUMOCOCCUS

Institution	Number of Cases								Mortality Percentage			
	Group I		Group II		Group III		Group IV		Group I	Group II	Group III	Group IV
	R*	D*	R	D	R	D	R	D				
Hospital of Rockefeller Inst. ...	32	9	40	16	14	15	58	10	22.0	28.6	52.0	14.7
Pennsylvania Hospital.....	23	9	19	7	1	2	30	4	29.0	27.0	67.0	11.0
Cook County Hospital <sup>34</sup> .....	37	13	18	7	1	4	23	8	26.0	28.0	80.0	26.0
Total.....	92	31	77	30	16	21	111	22	25.2	28.0	56.0	14.0
									General Mortality 25 per Cent.			

\* R = recovered; D = died.

Table 26, which was based on a study of 534 cases occurring in three clinics, each in a different city, shows that an average of 36 per cent. of all cases of lobar pneumonia due to the pneumococcus are caused by strains of this organism belonging to Group I. This percentage of incidence is greater than that of any of the other three groups; the incidence of Groups II and IV being approximately equal, whereas, infection with strains belonging to Group III is much more infrequent. On the other hand, reference to Table 27 shows that the mortality among cases of infection with Group III is much greater

34. Mathers, G.: Jour. Infect. Dis., 1915, **17**, 514.



than that caused by infection with members of any of the other groups, Group II ranking second in this respect, Group I third and Group IV last. These tables show clearly the importance of determining in every instance the particular group to which the infecting pneumococcus belongs when attempting to draw any conclusions from mortality statistics as to the value of any particular form of treatment in acute lobar pneumonia. Thus, if a particular series of cases studied were due, for the most part, to infection with organisms belonging to Group III, owing to the relatively high death rate (56 per cent.) in this group, the number of expected deaths would be greater than the number of such if the cases were due, for the most part, to organisms belonging to Group IV, in which the death rate is comparatively low (14 per cent.).

Although the number of cases reported in this communication is altogether too small to warrant final conclusions as to the value of the drug in the treatment of lobar pneumonia due to the various immunologic groups of pneumococci, nevertheless, it seems desirable for future reference to record the mortality results from this point of view. In thirty-two cases of lobar pneumonia the patients were treated with optochin; of these, twenty-four recovered and eight died, giving a mortality rate of 25 per cent. Thus, there is apparently no reduction in the death rate. We feel, however, that, in judging of the value of the drug, only those cases should be considered in which the patients were adequately treated, as far as our present knowledge of the subject permits us to decide. Accordingly, only those cases will be considered, from the point of view of mortality rate, in which the patients received sufficient optochin to insure the production of bactericidal action in the blood serum. We must, therefore, exclude from consideration those cases (Hosp. Nos. 2483 and 2573) in which for one reason or another the drug was discontinued shortly after administration had been begun, regardless of the ultimate outcome of the case; those cases (Hosp. Nos. 2581, 2508 and 2499) that failed to receive sufficient optochin per kilo of body weight per twenty-four hours to give rise to bactericidal action in the blood serum during the course of administration of the drug; one case (Hosp. No. 2466), that of a patient with vegetative endocarditis, who vomited frequently during the administration of the drug, so that undoubtedly much of the optochin was lost, and one case (Case 4, Hosp. No. 2659) in which crisis occurred before bactericidal action appeared in the blood. Finally, it was thought proper to exclude one case (Hosp. No. 2711) in which the patient received large doses of the type homologous serum intravenously, in addition to the optochin, and recovered. This case was one due to infection with a strain of pneumococcus belonging to Group I, in which group Cole<sup>35</sup> has obtained

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35. Cole, R.: New York Med. Jour., 1915, **101**, 1 and 58; Tr. Cong. Am. Phys. and Surg., 1916, **10**, 138.

very encouraging results from treatment with the type homologous anti-pneumococcus serum alone, on account of which fact the successful outcome of the case cannot be attributed mainly to the use of optochin. Those cases in which the patients received extract of antipneumococcus serum (Group II or Group III) in addition to the optochin, were not excluded from consideration, because the value of this agent in the treatment of cases of lobar pneumonia has not as yet been established, and also because in each instance the extract was not given until after the drug had been administered over a sufficiently long period and in sufficient amounts to produce bactericidal action in the patient's serum. Moreover, antiserums to Groups II and III are by no means as potent in protective power as the antiserum to Group I, and the Group II antiserum, which is more potent than that of Group III, has thus far proved disappointing in clinical practice. The pneumococcal action of the serum *in vitro* was shown not to have been enhanced by the administration of the serum extract.

In all, eight cases have been excluded; of these, four patients recovered and four died, one of the latter (Hosp. No. 2508, alcoholic, delirium tremens) in sixteen hours after the first dose of optochin. According to the revised statistics, twenty-four patients were considered to have been adequately treated with optochin, and of these, twenty recovered and four died, making a mortality rate of 17 per cent. The incidence of the various groups in this series of cases and the mortality rate of each group are represented in Tables 28 and 29, respectively.

TABLE 28.—INCIDENCE OF SEROLOGIC GROUPS OF PNEUMOCOCCI IN CASES OF LOBAR PNEUMONIA PATIENTS TREATED WITH OPTOCHIN

Group	Number of Cases	Per Cent.
II.....	10	42
III.....	7	29
IV.....	6	25
Unclassified.....	1	4
Total.....	24	

Table 28 shows that, of the patients that may be legitimately regarded as having been adequately treated with optochin, the majority of cases were caused by organisms belonging to Groups II and III. When it is recalled that, according to Table 27, the mortality among the patients whose cases were due to pneumococci belonging to these groups is greater than that among cases due to pneumococci of Groups

TABLE 29.—MORTALITY IN CASES OF LOBAR PNEUMONIA PATIENTS TREATED WITH OPTOCHIN, ARRANGED ACCORDING TO SEROLOGIC GROUPS

Group	Number of Cases	Recovered	Died	Mortality Rate, Per Cent.
II.....	10	9	1	10
III.....	7	5	2	29
IV.....	6	5	1	17
Unclassified.....	1	1	0	0
Total.....	24	20	4	17

I and IV, it will be seen that, although this series of cases is small, it nevertheless represents the severest types of infection with which one has to deal in cases of lobar pneumonia; therefore the drug may be said not to have had an easy trial. As explained earlier, only two patients belonging to Group I were treated with optochin. Table 29 shows the actual mortality rate in the various groups. Table 30 contrasts the actual mortality rate with the expected mortality rate in twenty-three treated patients, based on the figures given in Table 27, and also the number of deaths expected with that actually encountered (Hosp. No. 2672, unclassified, recovered, is excluded). A reduction in the mortality rate over that expected was observed in cases due to Groups II and III, and no reduction in cases due to Group IV. The number of cases due to strains belonging to Group I was too small to warrant recording, in this estimation, the results of the treatment in cases falling in this group. The total expected mortality rate, therefore, in this series of twenty-three cases was 33 per cent., and the total actual mortality rate was 19 per cent.

TABLE 30.—CONTRAST BETWEEN EXPECTED AND ACTUAL MORTALITY IN TWENTY-THREE PATIENTS TREATED WITH OPTOCHIN

Group	Number of Cases	Expected Mortality Rate, per Cent.	Actual Mortality Rate, per Cent.	Number of Expected Deaths	Number of Actual Deaths
II	10	23	10	3	1
III	7	56	29	4	2
IV	6	14	17	1	1
Totals.....	23	33	19	8	4

A summary of the complete series of cases is given in Table 32.



## DISCUSSION

The aim of specific chemotherapy in lobar pneumonia should be to destroy the pneumococci in the affected portions of the lung, to confine the pathologic process to the part of the lung involved, and to prevent the occurrence of empyema and of septicemia, as well as complications resulting therefrom; in short, to prevent direct or indirect extension of the morbid process from the focus of disease.

It is obvious that if a drug is to be used as a specific in the treatment of this disease it must possess a bactericidal action on pneumococci in the presence of body fluids; further, if it is to be given by mouth, it must be capable of being absorbed from the gastro-intestinal tract into the blood stream, and of being there maintained for an adequate period at a concentration sufficient to kill pneumococci without causing harm to the patient at the same time. To be of maximum value in the control of the disease, it should possess the property of passing through the capillary walls into the alveolar spaces and of penetrating the inflammatory exudate there encountered.

Optochin hydrochlorid fulfils at least some of these requirements under certain definite conditions. First, the drug, even in great dilution, possesses a marked bactericidal action on pneumococci *in vitro* in the presence of human blood serum and whole blood. In the second place, insofar as may be judged from the present study, which comprises too few cases to warrant final conclusions, it is capable of being absorbed from the gastro-intestinal tract into the blood stream in such amounts that a certain degree of pneumococcidal power may be maintained there for several days, provided the following conditions are fulfilled:

1. That the amount of the drug given per kilogram of body weight per twenty-four hours be at least 0.024 gm. (Amounts smaller than this, as low as 0.02 gm., may be sufficient to produce bactericidal action in the serum, but they cannot always be relied on to do so.)
2. That the size and spacing of the individual doses be suitably arranged.

Under such a regulation of dosage, however, amblyopia may result. This complication has occurred in 4.57 per cent. of all cases reported in the literature, and was generally recovered from; its occurrence in the future may possibly be lessened when the drug is given in accordance with a method such as that outlined above; there seems to be no doubt that some of the cases in which visual disturbances occurred received a larger amount of the drug than that necessary to insure the production of bactericidal action in the serum.

Whether or not the drug can pass through the capillaries of the pulmonary vessels and penetrate the fibrinous exudate in the alveolar

spaces cannot be stated. That it may pass through the capillary wall into a serous sac is demonstrated by one of our cases (Case 2, Hosp. No. 2705) which received optochin by mouth for several days, and in which it was possible to demonstrate in the pericardial fluid, obtained immediately after death, a bactericidal action on pneumococci in vitro as great in degree as that present in the blood serum shortly before death.

The available experimental evidence, therefore, apparently goes to show that optochin fulfils at least some of the requirements for its use as a specific chemotherapeutic agent in the treatment of acute lobar pneumonia, its chief drawback being the possibility that it may exert, in some instances, a toxic action on the eye. The ultimate decision as to the value of the drug in this disease, however, must rest on clinical experience based on its use in a large series of cases. In order to arrive at a scientific evaluation of its utility, it should be used systematically and according to a method that will insure the rapid production of bactericidal action in the serum and the maintenance of the same at a more or less constant level, within the limits of safety for the patient.

The rapid production and maintenance of the bactericidal action in the blood seem desirable in order that the infecting pneumococci may not acquire a condition of "fastness" to the drug, by virtue of which they become insusceptible to its action. That this consideration is not wholly theoretical is evidenced by the fact that pneumococci can acquire this property in vitro, and, further, by the demonstration that, in one instance (Case 17, Hosp. No. 2638), a strain of pneumococcus recovered from the heart's blood immediately after death was fast not only to concentrations of optochin much greater than that necessary to kill the strain obtained from the patient's blood on admission, but also to that concentration of optochin present in the blood of the same patient during the height of the treatment.

As already stated, the fact that the severity and mortality of the disease bear some relation to the particular group to which the infecting pneumococcus belongs, must be taken into account in an attempt to estimate the value of any treatment of lobar pneumonia. It should be emphasized that Groups II and III are the most virulent of the groups thus far recognized, and that the death rate in patients infected with strains belonging to these groups is greater than in the case of the other groups. Inasmuch as the majority of the cases treated in this study were due to infection with members of these two groups, the drug may be said to have had a severe test. In the revised figures, after exclusion of Case 12 (Hosp. No. 2711, recovered), in which other efficient therapeutic measures were employed, of one unclassified case (Hosp. No. 2672, recovered), and of seven cases (of which three



recovered and four died) where the optochin treatment for one reason or another was, on the basis of experimental or clinical observations, almost certainly inadequate, the expected mortality among twenty-three cases was calculated to be 33 per cent., whereas the actual mortality was found to be 19 per cent. It must, however, be again emphasized that the number of cases in the series is far too few to permit of final conclusions.

In this paper stress has been laid on the rapid attainment and maintenance in the circulating blood of a concentration of optochin sufficient to exert pneumococcidal action; further, it has been urged that the dosage of the drug should be regulated with this bactericidal action as a criterion, since this is the only objective method available for determining the optimum system of dosage. It must be borne in mind that a concentration of optochin in the circulating blood sufficient to exert bactericidal action *in vitro* may not, necessarily, be sufficient to influence favorably the course of the disease in the human body, for several reasons. It is probable that, for the drug to influence the course of lobar pneumonia to the greatest extent, it must penetrate the inflammatory exudate in the alveolar spaces and bring about the destruction of the pneumococci there present. To bring about the passage of the drug through the capillary walls into the exudate, a concentration of optochin in the blood stream in excess of that required to produce bactericidal action in the serum may be necessary. Whether or not this is the case cannot be stated on the basis of our present knowledge of the conditions governing the behavior of the drug in the human body; it is certain that the toxicity of the drug sets a limit to the concentration of optochin which may be attained in the serum in any given case. In one instance in the present study, as already stated, the drug was shown to be present in the pericardial fluid after oral administration. If this observation<sup>20</sup> should be confirmed in the future, the evidence will be in favor of the view that the drug can, ordinarily, make its way through the capillary wall into the alveolar spaces, although not necessarily into the exudate. Here it may be noted that empyema did not occur in any case of the present series.

The possibility of relative impermeability for optochin of the inflammatory exudate in the alveolar spaces suggests that the early use of the drug, before consolidation is complete, may be of importance, and that such a condition of comparative impermeability, occurring later in the disease, after complete consolidation has taken place, may be the reason why, according to several writers, better results are obtained in patients treated on or before the third day of the disease. In two instances in the present series, where treatment was started not later than the second day, rather striking results were obtained clinically, although the cases were due to infection with pneumococci belonging



to Group III, which shows a mortality greater than 50 per cent., and in which the disease generally runs a more severe course than in the case of infection with strains of the other groups.

Even if the drug is not capable of destroying all the pneumococci in the infected area when present in the blood in a concentration sufficient to produce bactericidal action in the serum *in vitro*, nevertheless, if it can prevent a spread of the disease process to uninfected areas and prevent the establishment of septicemia or metastatic complications, its use may be helpful; for experience seems to indicate that the occurrence of involvement of hitherto uninfected areas constitutes a source of danger to the patient.

The simultaneous use of optochin and antipneumococcus serum in the specific treatment of lobar pneumonia would seem to offer considerable hope. Until recently the treatment of lobar pneumonia with specific antisera has not been attended with striking results; this has probably been due, in part, to the failure to recognize the extent and significance of the immunologic differences existing among the pneumococci. Through the recognition of these differences it has become apparent that, in order to achieve any positive results in the treatment of lobar pneumonia with antisera, a serum corresponding to the particular group to which the infecting pneumococcus belongs must be employed. Recently Cole<sup>32</sup> has reported encouraging results obtained in a series of cases of lobar pneumonia due to infection with strains belonging to one of the immunologic groups (Group I) through the use of intravenous injections of the corresponding antiserum. In seventy-two consecutive patients thus treated the mortality was 8.3 per cent., whereas the death rate among untreated patients in this group is 25 per cent., so that specific serum therapy has undoubtedly succeeded in considerably lowering the death rate in at least one group of cases of lobar pneumonia. The use of antipneumococcus serum in Group II has, by itself, proved disappointing up to the present in the treatment of lobar pneumonia. Antipneumococcus serum has definite specific protective power against infection with a strain of the corresponding group in mice, and one of us (M.),<sup>30</sup> has shown that the protective value of the antiserum to Group II may be increased considerably if the animals receive a small dose of optochin simultaneously with the serum. This fact would seem to suggest that, in the specific treatment of lobar pneumonia, perhaps even more favorable results may be expected from the simultaneous employment of optochin and the type homologous antipneumococcus serum, than by the use of either of these remedies separately.

On the basis of the evidence presented in this communication the

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36. Moore, Henry F.: *Jour. Exper. Med.*, 1915, **22**, 389.

following suggestions are made for the use of the drug, although the observations are too few in number to permit of dogmatic statements.

1. It would seem, as far as present knowledge goes, that optochin hydrochlorid is best administered by the mouth in acute lobar pneumonia. (The drug is preferably given in capsules, because of its bitter taste.)

2. The drug should be administered in such amounts as will insure the rapid production and more or less constant maintenance, within the blood stream, of a concentration sufficient to exert bactericidal action on the pneumococci.

3. To achieve this result, the total amount of optochin given by the mouth per twenty-four hours should bear a definite relationship to the body weight of the patient, namely, at least 0.024 gm. of optochin hydrochlorid per kilogram of body weight. (It is possible that, after one or more days' administration of the drug, lesser amounts may suffice owing to retention or accumulation within the body.)

4. To secure the rapid production of bactericidal action in the blood stream the initial dose should be larger than the subsequent ones, the body weight relationship being at all times preserved.

5. The individual doses should be given regularly throughout the treatment and the intervals between the doses should, in general, not exceed two to three hours. Thus, in the case of individuals of average weight, say of 62 kg., 0.024 gm. per kilogram of body weight per twenty-four hours would mean a total of 1.5 gm. of the drug per twenty-four hours (Table 31).

TABLE 31.—SCHEME OF DOSAGE FOR INDIVIDUAL OF AVERAGE WEIGHT (62 KG.)

First 24 hours of treatment	Initial dose of 0.45 gm. Interval three hours Seven doses of 0.15 gm. each, with an interval of three hours between each two doses	Total, 1.5 gm.
Subsequent periods of 24 hours each	Ten doses of 0.15 gm. each, with an interval of about 2½ hours between each two doses	Total, 1.5 gm.

When the patient is below average size, the utilization of the same body weight relationship should insure the production of the bactericidal action in the blood stream, as shown above (Cases 10, Hosp. No. 2675, and 11, Hosp. No. 2626, p. 644); such patients require a smaller total quantity of optochin per twenty-four hours than patients of greater weight. For example, a patient weighing 42 kg. should require only 1.0 gm. of optochin per twenty-four hours. The size and spacing of the individual doses in such a case is indicated by the treatment of Cases 10 and 11 (Hosp. Nos. 2675 and 2626, Tables 13 and 14, pp. 644 and 645).

When the patient is above average weight, in order to preserve the

desired relationship between the amount of the drug and the body weight, more than 1.5 gm. must be given per twenty-four hours. Thus, a patient of 80 kg. would receive a total quantity of 1.9 gm. per twenty-four hours. Whether this comparatively large dose is safe or not, we cannot say. The possibility of untoward effects should always be remembered not only when such larger total daily doses are used in larger patients, but also in the case of small or average sized persons, even though the body-weight relationship be maintained. In the use of the drug impairment of vision should be carefully looked for and the drug should be discontinued, or at least reduced in amount, on its appearance. Daily ophthalmoscopic examinations might be advantageous.

Experiments *in vitro* have shown that the pneumococidal action of optochin is manifest in very high dilution, and that the concentration required for the production of bactericidal action is not much greater than that which produces inhibition of growth. If an average sized individual, say of 60 kg., receive 1.5 gm. of optochin, and a large individual, say of 80 kg., receive the same dose per twenty-four hours, the former would get 0.007 gm. of the drug per kilogram of body weight per twenty-four hours more than the latter, an amount which we should, from our experience of the drug *in vitro*, expect to be sufficient to replace mere inhibition by actual bactericidal action in the serum.

The present study comprises too few cases to permit of final decision regarding the optimum dosage of the drug or its value in the treatment of lobar pneumonia; these points can only be ascertained when a much larger series of cases is available for analysis.

#### CONCLUSIONS

1. Variations in the concentration of ethylhydrocuprein (optochin) hydrochlorid in normal horse serum are evidenced by corresponding variations in the degree of inhibitory and bactericidal power on pneumococci, when the drug is allowed to act on these micro-organisms in the test tube at 37 C.; the degree of the specific inhibitory or bactericidal power of optochin-containing serum gives an indication of the concentration of optochin present.

2. The members of a four-hour bouillon culture of pneumococcus are more resistant to the action of optochin than those of a twenty-hour culture (37 C.).

3. The use of "young" (four to six hours at 37 C.), actively growing bouillon cultures of pneumococci in bactericidal tests, affords a more delicate index of the differences in the degree of inhibitory or bactericidal action of different concentrations of optochin than does the use of older (twenty hours at 37 C.) cultures.



TABLE 32.—

Case No.	Hosp. No.	Age	Weight, Kg.	Group of Infecting Pneumococcus	Blood Culture Before Treatment	Day of Disease When Optochin Treatment Was Begun	Method of Dosage of Optochin in Periods of 24 Hours	Amount of Optochin, in Gm. per Kilo of Body Weight per 24 Hours (First Two Periods)	Total Amount of Optochin in Gm.	Duration of Treatment with Optochin, Days (24 Hrs.)	Duration of Disease, Days
	2477	26	52	II	Sterile	4	$3 \times 0.5$	0.0288	4.5	2.75	7
	2493	22	67.4	II	Sterile	3	$3 \times 0.5$	0.0222	6.0	4	5
	2521	34	57.4	II	Sterile	7	$10 \times 0.15$	0.0261	7.5	5	12
	2483	40	81.6	II	Sterile	3	$3 \times 0.5$	0.0183	3.0	2	8
9	2581	35	80	II	Sterile	3	$1 \times 0.5 + 7 \times 0.15$ ; $10 \times 0.15$ thereafter	0.0187	3.8	2.5	11
18	2573	16	52.6	II	Sterile	3	$3 \times 0.5$	0.0285	2	1.3	9
7	2611	60	58.4	II	Sterile	2	$1 \times 0.45 + 7 \times 0.15$ ; $10 \times 0.15$ thereafter	0.0256	5.7	3.75	?
	2586	26	65.4	Ii	Sterile	3	$1 \times 0.45 + 7 \times 0.15$ ; $10 \times 0.15$	0.0229	5.25	3.5	7
	2466	16	44.9	II	Positive; 40 col. per 1.0 c.c.	2	$3 \times 0.5$ ; $6 \times 0.25$ ; vomited frequently	?	?	2.0	4
11	2626	15	42.2	II	Sterile	6	$1 \times 0.3 + 9 \times 0.1$ ; $12 \times 0.1$ ; $8 \times 0.15$ thereafter	0.0284	5.65	4.6	11
6	2673	39	81.6	II	Positive	3	$1 \times 0.5 + 9 \times 0.15$ ; $13 \times 0.15$ ; $2(12 \times 0.15)$ ; $11 \times 0.15$	0.0226 0.0238	9.5	5.5	8
	2502	65	62	II a	Sterile	3	$10 \times 0.15$	0.0242	6.6	4	7
15	2604	20	55.4	II a	Sterile	4	$1 \times 0.15$ intramus. + $7 \times 0.15$ by mouth; $10 \times 0.15$ thereafter by mouth	0.0270	4.5	3	7
13	2658	29	66.6	II a	Sterile	2	$1 \times 0.5$ intramus. + $4 \times 0.25$ intramus.; $10 \times 0.15$ thereafter by mouth	0.0225	3.9	2.5	5
	2508	48	75 est.	III	Positive; 2 col. per 1.0 c.c.	4	At rate of $10 \times 0.15$	0.020 est.	1.2	0.75	5
8	2566	21	67.2	III	Sterile	2	$1 \times 0.45 + 7 \times 0.15$ ; $10 \times 0.15$	0.0223	3.9	2.75	6
	2550	63	73.3	III	Sterile	2	$1 \times 0.5 + 7 \times 0.15$ ; at rate of $10 \times 0.15$ thereafter	0.022	2.6	8	11
16	2582	48	45.3	III	Sterile	3	$1 \times 0.4 + 6 \times 0.15$ ; $2 (10 \times 0.15)$	0.0286 0.033	8.5	8	11

# SUMMARY OF CASES

Latest Time of Appearance of Bactericidal Action in Serum of Patients after Initial Dose of Optochin	Toxic Symptoms Referable to Optochin	Complications	Result	Occurrence of "Spread" During Treatment	Remarks
Not studied	None	None	Recovered	None	Physical signs persisted one week after temperature became normal
24 to 34 hours	Necrosis at site of subcutaneous injection	Serous pleurisy	Recovered	None	Extremely ill patient; temperature remained elevated several days owing to serous pleurisy; second dose of optochin vomited; fourth and sixth doses given subcutaneously; fifth given intramuscularly
8 to 23 hours	None	None	Recovered	None	Alcoholic; constant delirium
No bactericidal action	None	Pericarditis	Died, 8th day of disease	None	Drug was discontinued three days before death
No bactericidal action	None	None	Recovered	Spread	Optochin discontinued and "extract" of antipneumococcus serum (Group II) given subcutaneously; no apparent effect of optochin on disease; total "extract" = 103 c.c. = 583 c.c. whole serum
5.25 hours	Retinitis with temporary blindness	None	Recovered	None	Drug discontinued after onset of amaurosis; eighteen-hour culture used for tests
16 hours	Temporary deafness and tinnitus	None	Recovered	None	Delayed resolution; extract of antipneumococcus serum (Group II) subcutaneously commenced 46 hours after optochin was started; total "extract" = 40 c.c. = 220 c.c. whole serum
37.5 hours, "young" cult. (very heavy seeding); "old" cult., 9 hours	None	None	Recovered	None	"Extract" of antipneumococcus serum (Group II) subcutaneously on fifth, sixth and seventh days; total "extract" = 47 c.c. = 235 c.c. whole serum
Not studied	Nausea and vomiting	Endocarditis	Died, 4th day of disease	Spread	Progressive septicemia
17.5 to 24.5 hours	Temporary deafness	None	Recovered	None	Chronic otitis media on admission
12 hours	None	None	Recovered	Spread	Blood culture positive before treatment; sterile 24 hours after first dose of optochin; extract of antipneumococcus serum (Group II) intravenously commenced 36 hours after optochin was started; total "extract" = 155 c.c. = 620 c.c. whole serum
Not studied	None	None	Died, 7th day of disease	None	
42 hours	None	None	Recovered	None	Extract of antipneumococcus serum (Group II) started intravenously and subcutaneously 48 hours after first dose of optochin; total "extract" = 22 c.c. = 111 c.c. whole serum
18 hours	None	None	Recovered	None	Bactericidal action appeared at 19½ hours, then disappeared and reappeared at 28.5 hours
No bactericidal action in 17 hours	None	None	Died 17 hrs. after treatment began	None	Alcoholic; delirium tremens; restraint necessary
12 hours	None	None	Recovered	None	Bactericidal action disappeared when dosage was decreased
2.75 hours	Temporary tinnitus	None	Recovered	None	Physical signs persisted 2 days after temperature became normal; eighteen-hour old culture used in tests
1.75 hours	Temporary deafness	None	Recovered	None	Slow defervescence; eighteen-hour old culture used in tests; drug omitted for 13½ hours on fourth day of treatment

TABLE 32.—

Case No.	Hosp. No.	Age	Weight, Kg.	Group of Infecting Pneumococcus	Blood Culture Before Treatment	Day of Disease When Optochin Treatment Was Begun	Method of Dosage of Optochin in Periods of 24 Hours	Amount of Optochin, in Gm. per Kilo of Body Weight per 24 Hours (First Two Periods)	Total Amount of Optochin in Gm.	Duration of Treatment with Optochin, Days	Duration of Disease, Days
14	2593	37	53.8	III	Sterile	2	1 × 0.4 intramus. + 7 × 0.15 by mouth; 6 × 0.15 by mouth; 9 × 0.15 by mouth; 8 × 0.15 by mouth; 10 × 0.15 by mouth;	0.0269 0.0167 (0.0250) (0.0223) (0.0276)	7.9	6	7
5	2640	46	55.8	III	Sterile	4	1 × 0.45 + 7 × 0.15; 10 × 0.15 thereafter	0.0268	9.45	7	10
	2689	58	61	III	Sterile	2	1 × 0.45 + 7 × 0.15; 10 × 0.15 thereafter	0.0270 0.0245	7.65	5.5	8
2	2705	34	52.8	III	Sterile	2	3 × 0.45; 1 × 0.45 + 7 × 0.15; 2 (9 × 0.15); 10 × 0.15 thereafter	0.0255 0.0284	9.9	7	8
	2499	58	80.4	III	Sterile	4	10 × 0.15	0.0186	6.0	4	8
	2568	55	74.4	IV	Sterile	4	1 × 0.45 + 7 × 0.15; 10 × 0.15 thereafter	0.0201	4.5	4	8
1	2609	34	65.4	IV	Positive	5	3 × 0.5; 1 × 0.45 + 7 × 0.15; 10 × 0.15 thereafter	0.0229	3.6	2.5	8
	2634	42	70.3	IV	Sterile	6	1 + 0.45 + 7 × 0.15; 10 × 0.15 thereafter	0.0213	5.25	3.5	11
3	2639	41	72.8	IV	Sterile	2	11 × 0.15	0.0226	4.05	2.5	5
10	2675	15	89.1	IV	Sterile	4	1 × 0.3 + 7 × 0.1	0.0253	1.0	1	5
17	2638	55	42.8	IV	Positive	4	1 × 0.5 + 1 + 0.15 + 7 × 0.1; 3 (12 × 0.1); 7 × 0.1 + 3 × 0.15; 9 × 0.1, etc.	0.0315 0.028	8.75	8	12
4	2659	29	74.5	I	Sterile	6	12 × 0.15	0.0241	1.8	1	7
12	2711	21	57.4	I	Positive	4	1 × 0.45 + 8 × 0.15; 10 × 0.15 thereafter	0.0287 0.026	4.05	2.5	7
	2672	64	66	?	Sterile	6	1 × 0.45 + 7 × 0.15; 10 × 0.15	0.0227	7.8	5	12



—SUMMARY OF CASES

Latest Time of First Appearance of Bactericidal Action in Serum of Patients after Initial Dose of Optochin	Toxic Symptoms Referable to Optochin	Complications	Result	Occurrence of "Spread" During Treatment	Remarks
No bactericidal action in 63.75 hours	Deafness; tinnitus	None	Died, 9th day of disease	Spread	Apparently no effect on course of disease; blood culture positive before death; optochin stopped 30 hours before death
5.5 hours	None	None	Recovered	None	Consolidated lung resolved slowly
26 hours	Tinnitus; slight deafness; both temporary	None	Recovered	None	Eight hours elapsed between first and second doses of optochin; Extract of antipneumococcus serum (Group III) subcutaneously; first dose 60 hours after optochin was started; total "extract" = 20 c.c. = 200 c.c. whole serum
11 hours	None	Serous pericarditis	Died, 8th day of disease	Spread	Extract of antipneumococcus serum (Group III) intravenously after optochin had been administered for six days; total "extract" = 55 c.c. = 550 c.c. whole serum
Not studied	None	None	Died, 8th day	Spread	
68 to 92 hours	None	None	Recovered	None	After 24 hours of administration of optochin the drug was omitted for 20 hours and then resumed
11 hours	None	None	Recovered	None	Blood culture positive before treatment; sterile 24 hours after first dose of optochin
26 hours	Tinnitus; slight deafness; both temporary	None	Recovered	None	
16.5 hours	None	None	Recovered	None	
15.5 hours	None	None	Recovered	None	
11 hours	None	None	Died, 12th	Spread	Strain "fast" to optochin was recovered from blood, postmortem
18 hours	None	None	Recovered	None	Crisis 8 hours after commencement of optochin
2 to 4 hours	None	None	Recovered	None	Antipneumococcus serum (Group I) intravenously; first injection 24 hours after first dose of optochin; blood culture positive before and after 24-hour treatment with optochin; total serum given = 300 c.c.
24.5 hours after 1st dose; present first specimen	None	None	Recovered	None	

4. As judged from the present series of thirty-two cases, when optochin hydrochlorid is given by mouth to patients suffering from acute lobar pneumonia in such amounts that they receive at least 0.024 gm. per kilogram of body weight per twenty-four hours, and when the size and spacing of the individual doses are adequately regulated (see Dosage C, p. 627), a specific pneumococcal action appears in their blood within a few hours, and it can be maintained more or less constant for several days.

5. In order to maintain the bactericidal action in the blood at a constant level the intervals between the individual doses given by mouth should not ordinarily exceed about two and one-half to three hours (see Dosage C, p. 627).

6. When optochin is given by mouth according to such a scheme of dosage as outlined, the evidence points to some retention or accumulation in the blood of part of the drug absorbed.

7. Administration of optochin hydrochlorid by mouth appears to be more satisfactory than intramuscular administration. Further study of intramuscular administration appears to be desirable.

8. Pneumococci, not only *in vitro*, but also in the human body in patients treated with optochin, may acquire the property of more or less complete resistance or "fastness" to the drug.

9. Toxic symptoms, such as tinnitus, deafness, amblyopia or amaurosis (retinitis) may be observed in the use of the drug in man; they are generally transient. Retinitis, however, may result in more or less permanent impairment of vision.

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## AN EXPERIMENTAL TEST OF THE RELATION OF SEWAGE DISPOSAL TO THE SPREAD OF PELLAGRA \*

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### INTRODUCTION

In September, 1913, we<sup>1</sup> called attention to the apparent relation between the origin of new cases of pellagra and the employment of insanitary methods of sewage disposal. This relationship has been discussed in several subsequent publications of this commission, and we have offered the hypothesis that the methods of disposal of human wastes might prove to be a determining factor in the spread of pellagra in certain communities. A practical experiment was announced and described in our second progress report.<sup>2</sup> Since that time our epidemiologic observations on this particular point have been supported by other independent observers in Charleston, S. C.,<sup>3</sup> and in Nashville, Tenn.<sup>4</sup>

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\* From the Robert M. Thompson Pellagra Commission of the New York Post-Graduate Medical School and Hospital.

\* Read in part before the Society for Experimental Biology and Medicine, Nov. 15, 1916.

\* The epidemiologic surveys in 1915 and 1916, utilized in this paper, have been made since Dr. Garrison and Dr. Siler were recalled to active duty in the Medical Corps, U. S. Navy and U. S. Army, respectively. The paper itself has been written since that time. They are, therefore, not personally responsible for the observations of the last two years, for the compilation of the data, or for the deductions drawn from them.

1. Siler, J. F., Garrison, P. E., and MacNeal, W. J.: A Summary of the First Progress Report of the Thompson-McFadden Pellagra Commission. Read at the Special Pellagra Conference at Spartanburg, S. C., Sept. 3, 1913; Jour. Am. Med. Assn., Jan. 3, 1914, **62**, 8.

2. Siler, J. F., Garrison, P. E., and MacNeal, W. J.: The Relation of Methods of Disposal of Sewage to the Spread of Pellagra, *THE ARCHIVES INT. MED.*, 1914, **14**, 453; Second Progress Report, New York, 1915, p. 96.

3. Smith, W. A.: Epidemiology of Pellagra in Charleston, S. C. Read at the Third Triennial Pellagra Conference, Columbia, S. C., October, 1915.

4. Jobling, J. W., and Peterson, W.: A Preliminary Report on the Epidemiology of Pellagra in Nashville, Tenn., Jour. Infect. Dis., 1916, **18**, 501.



## THE EXPERIMENT

In the present communication we wish to present the results of the experiment begun in the community of Spartan Mills, Spartanburg, South Carolina, in 1913. This particular community had been a conspicuous endemic center of pellagra as long as the disease had been recognized in this region. Our records show that five new cases of pellagra were known to have appeared in this mill village previous to 1909, five in 1909, thirteen in 1910, twenty-three in 1911, eight in 1912 and thirty in 1913. In the fall of 1913 the installation of a water-carriage system of sewerage was begun, and the actual house connections were made as rapidly as the progress of the work would permit. One house was equipped in August, 1913; five houses in September, 1913; five blocks of houses in December, 1913; three blocks in January, 1914; three blocks in March; five blocks in April and seven blocks in May, 1914. A small group of houses, six in number, situated on very uneven ground at one corner of the mill property, was left without sewer connection. These houses were equipped with fly-proof pail closets. All the old, open, unscreened surface privies were demolished and removed. We had made a complete house-to-house survey of this community and of some of the adjoining groups of houses in 1913, and had accumulated rather extensive but, of course, much less complete records of the pellagrins there in previous years. The house-to-house survey was again made in 1914, in 1915 and in 1916, and in these years the survey was also extended to some of the neighboring districts.

In 1914 considerable attention was given by the Spartanburg Health Department to the privies everywhere in the city, and the owners of the houses adjacent to Spartan Mill, which is within the city limits, were compelled to make all privies fly-tight and leak-proof in that year. Subsequently, many of these privies have been maintained in a fairly sanitary condition, but others have fallen into a state of dilapidation, such that they are, from the point of view of disease dissemination, not at all different from the old surface privies. On the other hand, the sewered closets of the mill houses, which were occasionally stopped up or out of use because of ignorance of the population during the first year, have become more and more efficient as the people have become accustomed to their use and have recognized the ease with which they can be kept clean. It should also be mentioned that the houses on the mill property all belong to the mill company and are rented to their employees, who, as a rule, are not property owners. The houses on the neighboring property are privately owned and frequently occupied by the owners, and, if rented, are generally rented to people in better financial circumstances than the mill population.

## THE RESULTS

From 1908 to and including 1913, eight<sup>5</sup> new cases of pellagra appeared in the houses immediately adjacent to the mill property, which have been included in this survey. During the same time there arose eighty-three<sup>6</sup> new cases within the mill property. Table 1 shows the number of new pellagrins that appeared in each of these two sections in each year since 1908. The population living on the mill property was approximately 2,000 people, and that in the adjacent houses included in this survey approximately 300.

TABLE 1.—PELLAGRINS INCIDENT IN SPARTAN MILLS AND IN THE ADJACENT SURVEYED DISTRICT IN EACH YEAR.

	1908	1909	1910	1911	1912	1913	1914	1915	1916
In Spartan Mills.....	4	5	13	23	8	30	18	8	2
In adjacent district.....	0	2	0	1	3	2	3	2	4

During the year 1914, in which the sewer connections were completed, eighteen persons developed initial symptoms on the mill property. One arose in a house which was sewered in December, 1913, but just across the street from a house in which pellagrins were living, and which was not connected to the sewer until May, 1914. One arose in a house well within the district sewered in December, 1913. This patient was a married woman, whose mother, Pellagrin 237, had suffered from pellagra for many years. The mother lived in the neighboring unsewered district and was frequently visited by her daughter. Three cases appeared in a section which was sewered in January, 1914, eight arose in a section which was connected with the sewer in March, 1914, one in a section connected with sewer in April, and the remaining four in a section which was connected with the sewer in May, 1914. The installation of the sewers was probably too late to influence very much the pellagra incidence in 1914.

In the year 1915 there were eight new cases of pellagra found on the mill property. Two of these were children who had been living for at least two years in a district sewered in March, 1914. The father of one and the mother of the other were old pellagrins. In both these

5. This number does not include Pellagrins 91 and 991 with origin in 1911 and 1913, respectively. Both of these patients apparently contracted the disease while residing in this district, but moved away before the erythema appeared.

6. This number does not include Pellagrin 868, who had resided in Spartan Mills for about twelve years, but first developed the pellagrous erythema in 1913 while away on a visit.

children the diagnosis is somewhat doubtful, for they showed an eruption on the feet only, and this eruption has never recurred. Another case was in a woman who had lived in the sewered district since November, 1914. She gave birth to a child in June, 1915, and broke out with pellagra in July, 1915. Previous to November, 1914, she had lived in an unsewered mill in next-door relationship to a pellagrin. Another patient, Pellagrin 1282, was a feeble-minded woman, who denied all symptoms of pellagra, but showed a typical eruption in August, 1915. She may have had the disease previously, but has been recorded as a 1915 case. She lived with her mother, Pellagrin 940, in the sewered house since October, 1914, having previously lived in the unsewered section, where her mother contracted the disease in the summer of 1914. Another woman, Pellagrin 1336, had lived in the mill village since May, 1913, and suffered her first attack of pellagra in April, 1915. In February, 1915, she spent a week at her father's house in the country, her mother being severely ill with some gastrointestinal disorder at that time. Her father had been a pellagrin for several years and a sister, living at home, had her first attack in the previous summer. Pellagrin 1336, herself, developed pellagrous erythema of the hands about six weeks after her return to Spartan Mills. Another case was in a baby who came to this village in the latter part of January, 1915, and broke out with pellagra May 28, 1915. Her mother was an old pellagrin and was severely ill with a recurrent attack of the disease in April, 1915, and was committed to the State Hospital for the Insane about May 20. The house was in a filthy condition during the mother's illness. In an unscreened house next door to this one, Pellagrin 1279 had her initial attack June 15, 1915. She had lived there only since March. Before that she had lived for eighteen months in an unsewered section. Her daughter, aged 7, living with her, had been a pellagrin since 1913. The eighth patient, Pellagrin 1303, had lived in a neighboring unsewered mill village from November, 1914, to April, 1915, and she had shown indefinite symptoms of pellagra there in March, 1915. Her first erythema appeared early in June, 1915, about two months after coming to Spartan Mills.

In 1916 only two cases of pellagra are known to have appeared in the mill village. One woman, Pellagrin 1377, had her initial attack in June, 1916. She was an old resident and lived in a house at the edge of the sewered district just across the street from an unsewered house in which an old pellagrin resided. Pellagrin 1377 had borne a child in the latter part of April, 1916. The other patient, Pellagrin 1356, came to Spartan Mills in April, 1916, and developed her first erythema in that month. Previously she had been living with her daughter, Pellagrin 1167, in an unsewered house.



In this sewered district the number of new cases each year has shown a progressive diminution since 1913, being eighteen in 1914, eight in 1915 and two in 1916. The installation of the sewers could have had only a partial effect in 1914, because the connections were not completed until May of that year. In 1915 the incidence diminished very appreciably, but a number of new cases appeared among people who had moved into the mill village within a year. This influx was due to the very serious financial depression, and especially to the general depression in the cotton market from August, 1914, through 1915. Except for the two children in whom the diagnosis was doubtful, and the one woman, Pellagrin 1336, who had nursed her mother on her father's farm for a week in February, 1915, all the new pellagrins had moved within a year. In 1916 the result of the experiment is even more clearly evident. In this year only one new case appeared among the older residents on the mill property, and that was in a house at the extreme edge of the sewered district.

During the same years nine new cases of pellagra appeared in the small group of the more well-to-do population living in the adjacent partly sewered district which has been included in the survey. Three of these appeared in 1914. Pellagrin 1389, a contracting carpenter in very comfortable financial circumstances, suffered his first attack in 1914 in a house next door to pellagrins living on either side in unsewered houses. He had been living in the same house for several years. Pellagrin 940, an old woman, suffered her first attack in 1914 in a house next door to an unsewered house in which two pellagrins were living. Pellagrin 1289, a young girl, had her first attack while living with her pellagrous mother in an unsewered house. In 1915, after the general enforcement of the sanitary ordinance in regard to privies in Spartanburg City, only two new cases of pellagra appeared in this district. One was a man, Pellagrin 1314, living in an unsewered house with his wife, an old pellagrin. The other patient was a woman, Pellagrin 1285, who had moved about a good deal. She lived with her mother, Pellagrin 912, until the summer of 1914, in a sewered house just across the street from an unsewered house in which an active pellagrin resided until October, 1914. This patient, Pellagrin 1285, moved to the unsewered district outside the mill property Jan. 1, 1915, and into a house just vacated by a pellagrin. She gave birth to a child Feb. 14, 1915, and had her initial erythema in May, 1915.

In 1916 there were four new cases of pellagra in this district, three of them in one little focus in two houses immediately adjacent to a dilapidated and very dirty surface privy, which was used daily by old pellagrins, and one of them in a sewered house in which an old pellagrin resided at the time.

TABLE 2.—PELLAGRINS RESIDING IN SPARTAN MILLS IN EACH YEAR CLASSIFIED ACCORDING TO MANIFESTATIONS OF THE DISEASE AND LENGTH OF RESIDENCE

Year	With Indefinite Record		Without Recurrence		With Recurrent Attack		With Initial Attack	
	Old Residents	Recent Arrivals	Old Residents	Recent Arrivals	Old Residents	Recent Arrivals	Old Residents	Recent Arrivals
1908	0	0	0	1	1	0	3	1
1909	1	1	1	0	3	1	2	3
1910	0	0	1	1	3	0	9	4
1911	2	0	2	1	8	3	13	10
1912	1	1	9	0	17	3	4	4
1913	1	1	13	1	21	5	16	14
1914	1	1	18	7	27	13	7	11
1915	1	0	32	10	26	14	3	5
1916	3	0	49	6	15	6	1	1

Table 2 shows the number of cases of pellagra known to have resided in Spartan Mills in each year from 1908 to 1916, inclusive, classified according to initial attack, recurrent attack, absence of recurrence and lack of definite record. Each of these classes has been subdivided into two groups; one group, designated as old residents, being made up of those who had been living in the village for at least a year, and a second group comprising those who had moved into the village within the year.

Table 3 shows in a similar way the pellagrins known to have resided in the surveyed adjacent district. The data of these tables are pre-

TABLE 3.—PELLAGRINS RESIDING IN HOUSES ADJACENT TO SPARTAN MILLS IN EACH YEAR CLASSIFIED ACCORDING TO MANIFESTATIONS OF THE DISEASE AND LENGTH OF RESIDENCE

Year	With Indefinite Record		Without Recurrence		With Recurrent Attack		With Initial Attack	
	Old Residents	Recent Arrivals	Old Residents	Recent Arrivals	Old Residents	Recent Arrivals	Old Residents	Recent Arrivals
1908	0	0	0	0	1	0	0	0
1909	0	0	0	0	0	1	2	0
1910	0	0	0	0	3	0	0	0
1911	0	0	0	0	3	2	0	1
1912	0	0	0	0	4	3	1	2
1913	1	0	2	4	6	2	2	0
1914	1	0	2	1	2	1	2	1
1915	0	0	1	0	6	0	1	1
1916	0	0	6	2	1	4	2	2

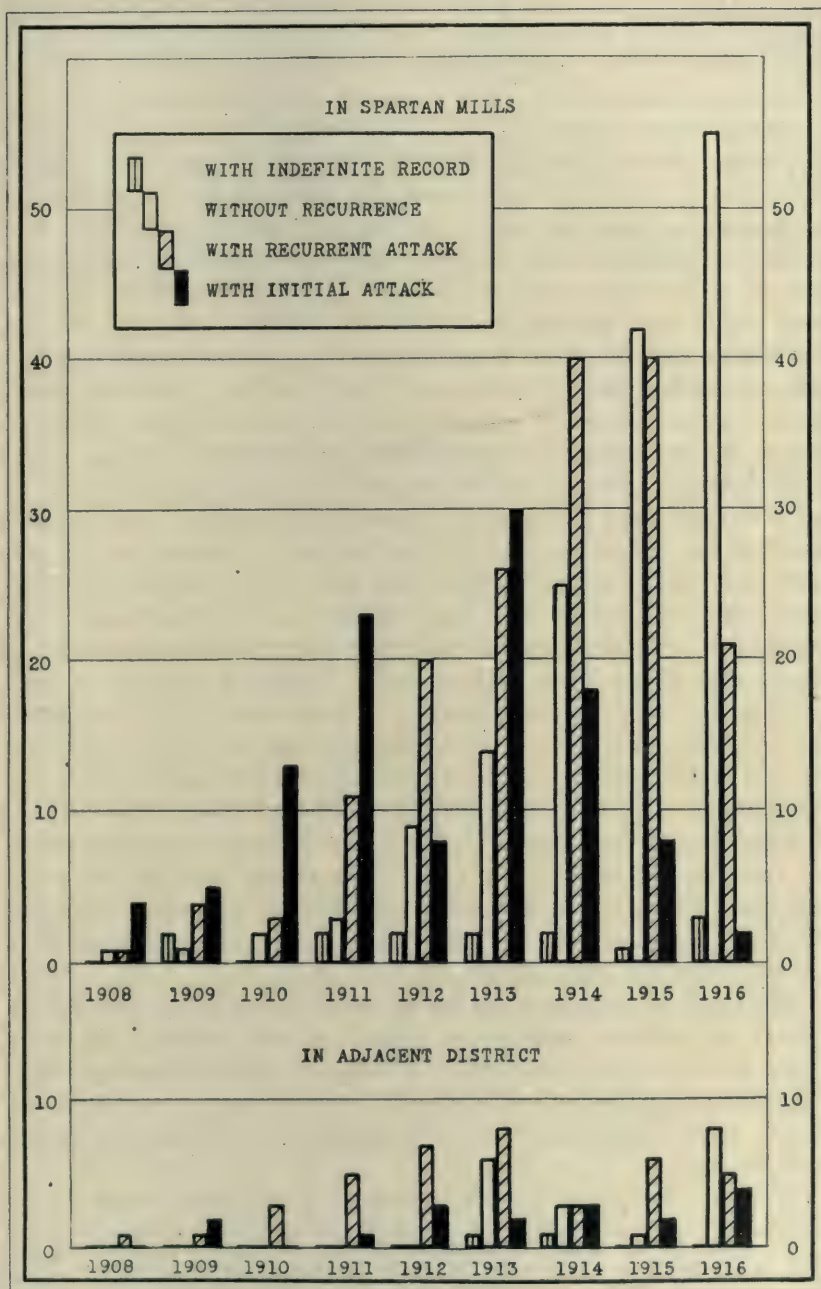


Fig. 1.—The total number of pellagrins residing in Spartan Mills and in the adjacent surveyed district in each year.



sented in graphic form in Figures 1 and 2. The former shows the total pellagrins of each class present in each year in the mill village and in the adjacent district, and Figure 2 shows in a similar way the old residents, excluding all who had moved in within the year.

In the charts one can see that the number of newly incident cases of pellagra reached the maximum in 1913, after which the number diminished rapidly. We believe that this reduction may be reasonably ascribed to the improved sanitation instituted in 1913 and 1914. The number of pellagrins who suffered recurrent attacks increased each year, to reach its maximum in 1914, in which year forty of the residents within the mill village suffered from recurrent pellagra. In 1915 the number with recurrent attacks of the disease was the same, but in 1916 there was a marked diminution in the number of recurrent cases. The amount of recurrent pellagra seems not to have been influenced directly by the change in sanitation. The diminution in this group would appear to be due in part to the falling off in the number of newly incident cases in the years immediately preceding. As we have shown in an earlier paper<sup>7</sup> in this series, the proportion of old pellagrins who suffer recurrence of the disease tends to diminish progressively with the lapse of time, and inasmuch as only eight new cases appeared in 1915, the recurrences in 1916 could not be very numerous unless cases dating from 1914 or before furnished the bulk of such recurrences. The diminished number of recurrences in 1916 has probably been due in part also to the increased prosperity of the population and to a considerable degree to the free dinners provided for these old pellagrins by the United States Public Health Hospital. The number of pellagrins without recurrent attack has increased progressively to the maximum of fifty-five in 1916. This shows that the pellagrous population has not been decreased especially by emigration, but that the patients have remained in the village for the most part and have outlived the active stage of the disease.

The situation brought about by the installation of the sewer system appears to be closely analogous to the condition previously observed by us<sup>2</sup> in the Newry Mill Village, a situation such that pellagra may continue to recur in those patients who have previously contracted the disease, but in which new cases of pellagra do not arise in any appreciable number.

The data for pellagra in the immediately adjacent district are of interest as a control study. The population of this district was about one-sixth as large as that of the mill village. From 1908 to 1913, inclusive, there had been eight instances of initial attack of pellagra

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7. Siler, J. F., Garrison, P. E., and MacNeal, W. J.: The Subsequent History of Pellagrins in Spartanburg County, S. C., Who Survived the Initial Attack, *THE ARCHIVES INT. MED.*, 1916, **18**, 340.

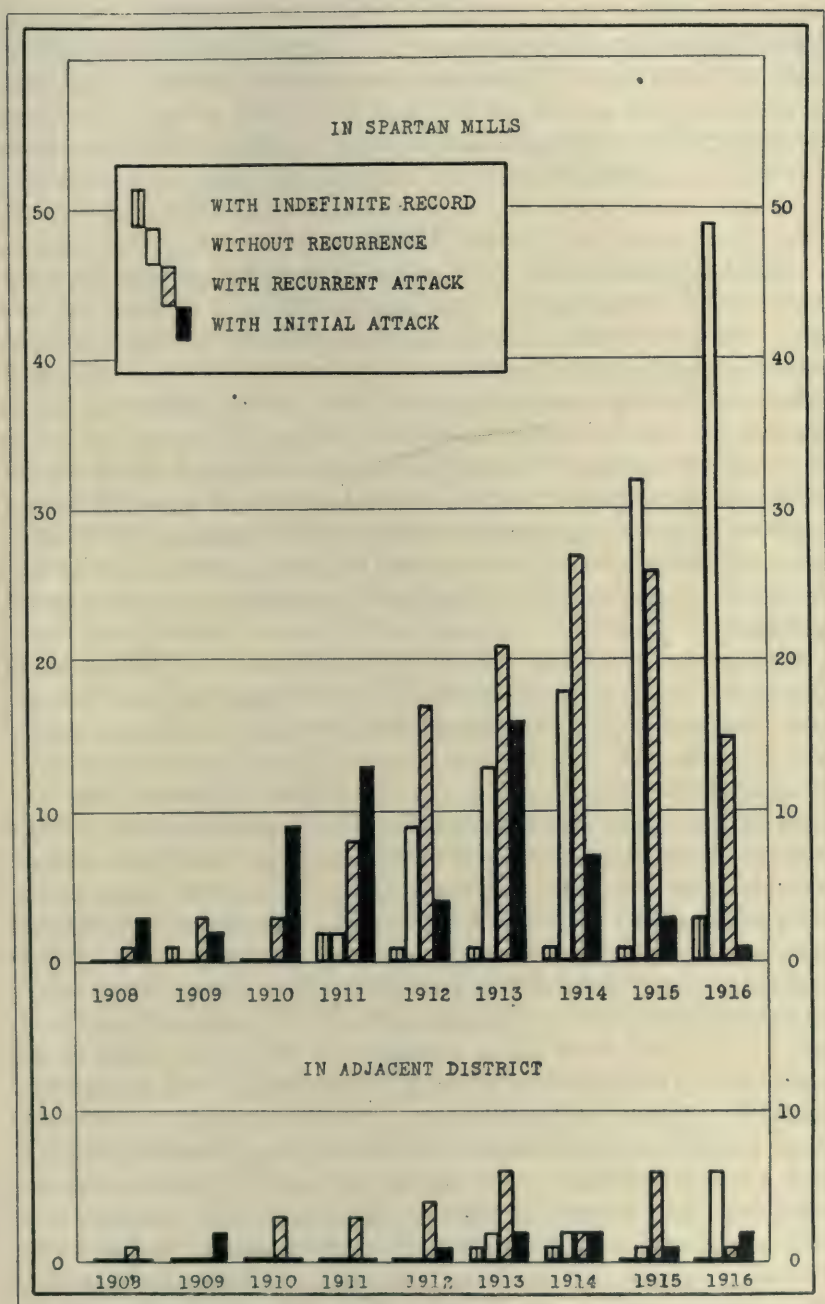


Fig. 2.—The number of pellagrins among old residents in Spartan Mills and in the adjacent surveyed district in each year.

here, while eighty-three cases had arisen in the mill village itself. In 1914 there were three new cases in this control district and eighteen in the mill village; in 1915 two here and eight in the mill village, and in 1916 four here as against only two in the mill village. It will be noted that pellagra has continued to spread in this district adjacent to the mill village since 1913 at about the same rate as it did before.

#### COMMENT

The diminution in new cases of pellagra in this community from thirty in 1913 to eighteen in 1914, eight in 1915 and two in 1916, has been very remarkable, and the obvious cause of this improvement would appear to be the intentional experimental factor, namely, the installation of the sewerage system. It is necessary, however, to examine critically the experiment and to give full consideration to other possible factors which may have entered into and influenced the result. Especially important in this respect would seem to be the condition of general prosperity and the possible changes in food habits of the population, for it is well known that both incident and recurrent attacks of pellagra tend to be more prevalent among the poor and ill-nourished.

The general prosperity may first be considered. In 1913 and 1914 these people were in better financial circumstances than in 1911 and 1912. In 1915 they were suffering from the general business depression, which became serious about Aug. 1, 1914, too late, however, to be effective in the pellagra season of 1914. This depression caused an influx of the poorer farming population to the mill, where work could be obtained although wages were low. The general financial condition of the population in 1915 was much worse than in 1914 and probably worse than in 1911 or 1912. In 1916 this situation had been relieved and the demand for labor elsewhere had brought about a slight increase in wages in the mill in order to retain the workers. Financial conditions in 1916 were much better than in 1915 and probably better than in 1913 and 1914. The influence of this factor is apparently manifested by the smaller number of recurrences in 1916 as compared with 1915. It is necessary, however, to keep in mind that a reduction in the number of newly incident cases in one year necessarily tends to bring about a reduction in the number of recurrent cases in the next subsequent year or two, so that the small number of recurrences in 1916 may be partly due to smaller crops of new cases in 1914 and 1915. Granting, for the moment, that increased prosperity has caused a reduction in recurrent cases from forty in 1914 and forty in 1915 to twenty-one in 1916, it is evidently quite impossible to ascribe to it the decrease in newly incident cases from thirty in 1913 to eighteen in 1914, when the recurrent cases increased from twenty-six to forty, or



the further decrease to eight incident cases in 1915 while the recurrences still remained at forty. Furthermore, in 1916 the newly incident cases were reduced almost to the vanishing point while the recurrent cases were only about 50 per cent. less than the largest number ever observed. The reduction in the incident cases cannot, therefore, be satisfactorily ascribed to the improved financial conditions.

Another factor which requires consideration is the possible change in diet of the population, dependent on educational propaganda resulting from the investigations of pellagra, and on the presence of the pellagra hospital located within this mill village. This hospital was conducted by our commission in 1913 and was taken over by the United States Public Health Service in 1914. The value of improved nutrition in the prevention and treatment of pellagra has been preached to this population by us since 1912, and since the fall of 1914 the Public Health Service has provided a free dinner for all known pellagrins who would come regularly to partake of it. This food was provided only for pellagrous individuals and not for the general population. The educational campaign has also reached especially the pellagrins, but of course to a less degree the general population. It is perhaps justifiable to credit, in part, to this campaign of dietary improvement the progressive increase since 1912 in the number of old pellagrins without recurrence present in this community and the actual reduction in recurrent attacks observed in 1916, especially the latter, because of the improved diet actually given to many of the old pellagrins. An attempt to ascribe the diminution in newly incident cases to improved dietary meets at once with difficulties of the same nature as those mentioned in the preceding paragraph. The diet has been applied directly and definitely to the old cases of the disease with the result that the recurrences have been reduced from twenty-six in 1913 to twenty-one in 1916, while among the general population, on whose diet the effect of this factor was only indirect and probably very slight, the number of incident cases of pellagra has diminished from thirty in 1913 to two in 1916.

When, therefore, the influence of these general factors is examined and measured by the behavior of the old cases of pellagra, it is found quite inadequate to explain the diminution in the number of newly incident cases of pellagra, and, therefore, quite inadequate to have influenced materially the result of the main experiment. A further light is thrown on the matter by the control group of population in the adjacent surveyed district.

The people in this district adjacent to Spartan Mills were influenced also by the general prosperity, by the educational propaganda and by the free dinners. The eight pellagrins in this district who escaped recurrence in 1916 may perhaps be reasonably credited in part to these

factors. It is, however, very significant that in spite of these general influences new cases of pellagra were as numerous as ever in 1916 in this district. In fact, more new cases occurred here in 1916 than in the enormously greater population of the mill village itself.

This experiment at Spartan Mills is, of course, only one experiment, and we realize that sweeping conclusions cannot justly be drawn from any single experiment in which various uncontrolled and unknown factors may have entered. Nevertheless, we feel justified in pointing out that the result of the experiment is fully in accord with the hypothesis announced at its inception, as a test of which it was undertaken. Furthermore, the outcome of this experiment considered together with the large mass of epidemiologic evidence, previously presented by us and confirmed by other independent observers, seems very convincing. We feel warranted in continuing to recommend most heartily the installation of sanitary systems of sewage disposal as an important means of restricting the spread of pellagra.

#### SUMMARY

1. Subsequent to the installation of a water carriage system of sewage disposal at Spartan Mills, the community has been transformed from a pellagra focus to a community in which pellagra no longer spreads.
2. Old cases of pellagra have continued to recur in this community, although a distinct decrease in them has occurred in the third year after the installation of the sewers.
3. The installation of sanitary systems of sewage disposal is recommended as a measure for the restriction of the spread of pellagra in the general population.

## INTESTINAL EOSINOPHILIA, WITH REPORT OF A CASE \*

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SAN FRANCISCO

The occurrence of eosinophilic cells or Charcot-Leyden crystals in the mucus of stools has been occasionally reported almost ever since Ehrlich first described the eosinophil in 1879. They have been found with greatest frequency in cases of parasitic disease, especially amebiasis and hookworm disease, and in the latter condition the finding has been reported to be almost a constant one. It has also been known for a long time that they may occur in certain non-parasitic conditions, notably mucous colitis.<sup>1</sup> But the great variability of the intestinal eosinophilia in cases otherwise entirely similar has made the finding rather a clinical enigma than a sign of any diagnostic importance. For this reason Schmidt<sup>2</sup> has considered it inadvisable to attempt any grouping of cases on this basis. In his opinion eosinophils may occur in varying numbers in intestinal mucus in almost any type of colitis. In a few cases, however, where the rectum and sigmoid have been particularly involved, other investigators have attempted to establish a type of intestinal eosinophilia as a clinical entity.

Neubauer and Stäubli<sup>3</sup> in 1906 reported seven cases in which either eosinophils or Charcot-Leyden crystals were noted in the stools. Of these, three were cases of acute diarrhea of unknown cause, clearing up promptly under hospital care. The stools all contained Charcot-Leyden crystals, and in one case many eosinophils. No parasites or ova were found, and there was no blood eosinophilia in any of the cases. The remaining four cases were of long standing—a few months to a few years—with a history of alternating constipation and diarrhea, and with negative physical findings. All the cases occurred in young adults. In the stools were found mucus, some blood, Charcot-Leyden crystals and eosinophils; no parasites or ova. There was a distinct

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\* Submitted for publication Jan. 11, 1917.

\* From the Medical Division of the Stanford Medical School.

1. Bäumler: Ein weiterer Fall von hochgradiger Anämie, *Cor.-Bl. f. Schweiz. Aerzte*, 1881, **11**, 10. Nothnagel: Beiträge zur Physiologie u. Pathologie des Darms, 1884. Akerlund: Studien über Enteritis membranacea, *Arch. f. Verdauungskr.*, 1896, **1**, 396.

2. Schmidt: Die Fäzes des Menschen, 1905, p. 86. Von Emden: Ueber eosinophile Zellen in den Darminhoud, *Abstr., Zentralbl. f. inn. Med.*, 1907 **24**, 872.

3. Neubauer and Stäubli: Ueber eosinophile Darmerkrankungen, *Münche. med. Wchnschr.*, 1906, **53**, 2380.



blood eosinophilia in three of the cases, in one case as high as 15 per cent., and this patient showed a marked secondary anemia. Proctoscopic examination showed a normal rectum in one case, but in the other three they found a much inflamed mucosa over which were scattered small areas of grayish exudate a few millimeters in diameter. Occasional erosions were noted, but no true ulceration. Microscopic examination of the exudate showed large numbers of eosinophilic cells. They consider the three cases with proctoscopic lesions to be instances of a clinical syndrome which they characterize as "eosinophilic proctitis," and that the condition is due to a constitutional anomaly.

Fricker<sup>4</sup> in 1907 reported another case occurring in a woman of 35 who had suffered from constipation her whole life until five weeks before coming to the hospital, when she began to have diarrhea, with a little blood at times. Physical examination was negative. Repeated examination of the stools showed some blood, much mucus, containing many eosinophils, and Charcot-Leyden crystals; no parasites or parasitic ova. Proctoscopy revealed a diffusely reddened rectal and sigmoid mucosa, thinly blood-streaked here and there, with a few small discrete areas of mucopurulent exudate. No blood examination was made. Fricker considers the case to be entirely similar to those reported by Neubauer and Stäubli. He also reports another case of intestinal eosinophilia in a child of 2½ years, with periodic diarrhea, without apparent cause. During one of these attacks the stools contained many small masses of pus the cells of which were mostly eosinophilic. The ova of trichuris trichiura were also found, and this parasite was considered to be etiologic.

Komarowsky<sup>5</sup> in 1910 reported two cases with diarrhea lasting several months. Physical findings were unimportant. The stools contained some blood and a moderate amount of pus, composed almost entirely of eosinophils. There was no increase in blood eosinophils in either case. The proctoscope in one case showed an edematous mucosa, somewhat eroded, with a few islands of loose mucopurulent exudate; in the other case there was a thick mucopurulent exudate throughout the ampulla recti.

Wiener<sup>6</sup> in 1912 searched for eosinophils in the mucus of seventeen cases of chronic proctitis. They were present in varying amount in seven of the cases, but were not associated with characteristic proctoscopic findings. The blood eosinophils were above 5 per cent. in only

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4. Fricker: Ueber zwei Fälle von Darmeosinophilie, München. med. Wchnschr., 1907, **54**, 260.

5. Komarowsky: Ueber lokale Eosinophilie und eosinophile Darmerkrankungen, Arch. f. Verdauungskr., 1910, **16**, 74.

6. Wiener: Ueber Eosinophilie des Darmschleims, Berl. klin. Wchnschr., 1912, p. 258.

two of the cases, and were highest (8 per cent.) in the case having the greatest number of eosinophils in the mucus.

Our attention has recently been drawn to the subject by the following case in the medical wards of Lane Hospital.

#### REPORT OF CASE

*History.*—G. F., Italian cook, aged 40, entered the hospital Aug. 21, 1916, complaining of pain in the right side of the back, and alternating constipation and diarrhea. The family history was unimportant. He had had probable pleurisy ten years previously, with cough and night sweats; is usually moderately constipated; for the previous five years he had had occasional pain in the right back, at times sharp, and increased by exercise.

The previous few months he had had occasional attacks of diarrhea alternating with constipation. During the attacks of diarrhea the bowels moved five or six times a day, and he frequently noticed masses of mucus in the stool, and at times blood.

*Examination.*—Examination on admission showed a well-nourished man of good color; heart and lung findings normal; spleen just palpable; nothing otherwise abnormal in abdomen; prostate small, tender; many pus cells in prostatic fluid. Urine: specific gravity 1.020, no albumin or casts. Red blood count 3,970,000; hemoglobin 50 per cent. Dare; white blood count 8,500: polymorphonuclears, 28 per cent.; lymphocytes, 20 per cent.; large mononuclears, 4 per cent.; eosinophils, 47 per cent. A stool following castor oil contained much mucus and red blood. Microscopically a very few ova of trichuris trichiura were found after extensive search with special methods. Wassermann negative. The proctoscope inserted 23 cm. showed the mucous membrane of the rectum everywhere covered with glairy mucus, pus and bright red blood. No areas of ulceration were seen. Smears of the pus showed a large percentage of eosinophils. No Charcot-Leyden crystals were seen. A small bit of necrotic tissue found in the stools was sectioned, and showed a chronic inflammation, with large numbers of eosinophils in the mucosa and submucosa.

The patient remained in the hospital for about one week, with little change in his condition, and has since been lost sight of.

This case falls readily into the group of intestinal eosinophilia with definite proctoscopic findings, but differs from the type of eosinophilic proctitis reported by other observers mainly in the marked blood eosinophilia, and in the finding of trichuris ova in the stools. With regard to the latter, inasmuch as there was apparently a very mild infection with this parasite, and as it is not known to produce rectal lesions or such a degree of blood eosinophilia, we are not inclined to attach great importance to the finding, although, as already noted, trichuris was considered to be causative in one of Fricker's cases.

Some light has perhaps been shed on the etiology of this condition, and on the question of eosinophilia in general, by the recent studies of the relation of eosinophilia to the anaphylactic state. It has long been recognized that there is a clinical relationship between asthma, hay-fever, certain skin affections, and the so-called neurotic bowel conditions characterized by the passage of large amounts of mucus. The so-called exudative diathesis, as outlined by Czerny and amplified by



Strümpell and others,<sup>7</sup> includes this group of conditions, and with the rise of the conception of anaphylaxis and its application to clinical matters, the view has become more and more prevalent that these conditions may be due to the effects of parenteral protein.<sup>8</sup> And since they are all frequently associated with an eosinophilia, it was reasonable to suspect that eosinophilia might be one of the manifestations of anaphylaxis. It has been known for many years that eosinophilia occurs at times following the injection of tuberculin<sup>9</sup> and of therapeutic serums,<sup>10</sup> but only recently have attempts been made experimentally to relate eosinophilia with anaphylaxis.

Schlecht<sup>11</sup> produced anaphylactic shock in experimental animals, and reported marked increase in blood eosinophils in the animals surviving the shock. He also reported eosinophilia following daily injections of protein. Confirmations of this work have been reported by Schittenhelm,<sup>12</sup> Weinberg and Seguin,<sup>13</sup> and in this country by Herrick,<sup>14</sup> although since the latter worked with ascaris extracts, the rôle of anaphylaxis is perhaps not so clear. If we can accept the reported experimental results of these investigators, certainly Herrick's remark is conservative, that "it is tempting to conclude that hypereosinophilia is the result of sensitization to alien protein." In connection with the subject of intestinal eosinophilia, the statement of Schlecht and Schwenker,<sup>15</sup> that the anaphylactic bowel in the dog shows an intense local eosinophilia, is of interest. These experiments clearly suggest the possibility that both the local and blood eosinophilia in patients such as ours may be due to the parenteral digestion of protein by the patient, though we have no knowledge of the nature or portal of entry of such protein.

Sacramento and Webster Streets.

7. Czerny: Die exsudative Diathese, *Jahrb. f. Kinderh.*, 1905, **61**, 199.  
Strümpell: Ueber das Asthma bronchiale und seine Beziehungen zur sogenannten exsudativen Diathese, *Med. Klin.*, 1910, p. 889.

8. Meltzer, S. J.: Bronchial Asthma as a Phenomenon of Anaphylaxis, *Jour. Am. Med. Assn.*, 1910, **55**, 1021.

9. Zappert: Ueber das Vorkommen der eosinophilen Zellen im menschlichen Blute, *Ztschr. f. klin. Med.*, 1893, **23**, 227.

10. Schlecht: Ueber die Einwirkung von Serum-Injectionen auf die Eosinophilen und Mastzellen, *Deutsch. Arch. f. klin. Med.*, 1910, **98**, 308.

11. Schlecht: Ueber experimentelle Eosinophilie nach parenteraler Zufuhr artfremden Eiweisses, *Arch. f. exper. Physiol. u. Pathol.*, 1911, **67**, 137.

12. Schittenhelm: Weichardt u. Grisshammer. Eiweissumsatz und Ueberempfindlichkeit, *Ztschr. f. exper. Path. u. Therap.*, 1912, **10**, 412. Ahl and Schittenhelm: Ueber experimentelle Eosinophilie nach parenteraler Zufuhr verschiedener Eiweissstoffe, *Ztschr. f. d. ges. exper. Med.*, 1913, p. 111.

13. Weinberg and Seguin: Recherches biologiques sur l'eosinophilie, *Ann. de l'Inst. Pasteur*, 1914, **28**, 470.

14. Herrick: Experimental eosinophilia with an Extract of an Animal Parasite, *THE ARCHIVES INT. MED.*, 1913, **11**, 165.

15. Schlecht and Schwenker: Ueber lokale Eosinophilie in den Bronchien beim anaphylaktischen Meerschweinchen, *Arch. f. exper. Physiol. u. Pathol.*, 1912, **68**, 163.



# THE SIGNIFICANCE OF EMBRYONAL FAT CELLS IN CERTAIN PATHOLOGIC CONDITIONS \*

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There are two outstanding theories concerning the histogenesis of the fat cell — one, that it is a cell set apart in early life for the specific purpose of producing fat, primitive fat organs, so-called, being found in various localities in the form of small groups of stellate or spindle cells richly permeated by capillary vessels. The cytoplasm of the primitive fat cell is finely granular and stains pinkish with eosin. As development proceeds the cell accumulates fat granules which fuse into globular shape, the cell itself becomes polygonal, then spherical in outline, the nucleus is peripherally displaced and finally compressed against the cell membrane, and the cytoplasm is reduced to a minimum. The other theory accounts for the origin of the fat cell on the basis of metaplasia of the fibroblast with the accumulation of fat within its body, and it is assumed that the fibroblast, when it fails to take on the specialized function of the fat cell, follows its natural inclination and develops into connective tissue.

It appears to us to be probable that both methods of cellular lipogenesis are effective, as shown by the fact that in certain pathologic conditions the primordial fat organs are noticeably increased in size, due to hyperplasia of their cellular constituents, and that, inversely, in many cachectic subjects, particularly in the epicardial and perirenal tissues, the fat disappears from the cell and the cytoplasm is replaced by serous fluid, the cell reverting to the polygonal form of the embryonal unit — the so-called serous atrophy of fat. Identical reversionary changes may be produced in the cervical fat tissues of the pig by prolonged fat starvation,<sup>1</sup> but, if the animal be given carbohydrate, the fat is replaced in the cell body. In other circumstances, notably in chronic productive inflammatory lesions attended by overproduction of fibroblasts, the transformation of young connective tissue elements into cells indistinguishable from those of the primitive fat organs can actually be observed in suitably stained microscopic preparations.

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1. Herter: Jour. Exper. Med., 1898, **3**, 293.

Up to, and even for a considerable period after birth, small groups of fat cells, collectively known as primitive fat organs, are to be found over a wide distribution. They may be so minute as to escape detection by the unaided eye, or they may be apparent in the form of small, rounded or oval, stellate, or otherwise angulated bodies whose color, depending on the degree of vascularity, varies from a yellowish-brown to a bright cherry-red. They occur with noticeable frequency around the kidneys and suprarenal capsules, in the loose connective tissues of the precordium, and in the fascial planes of the neck and interscapular

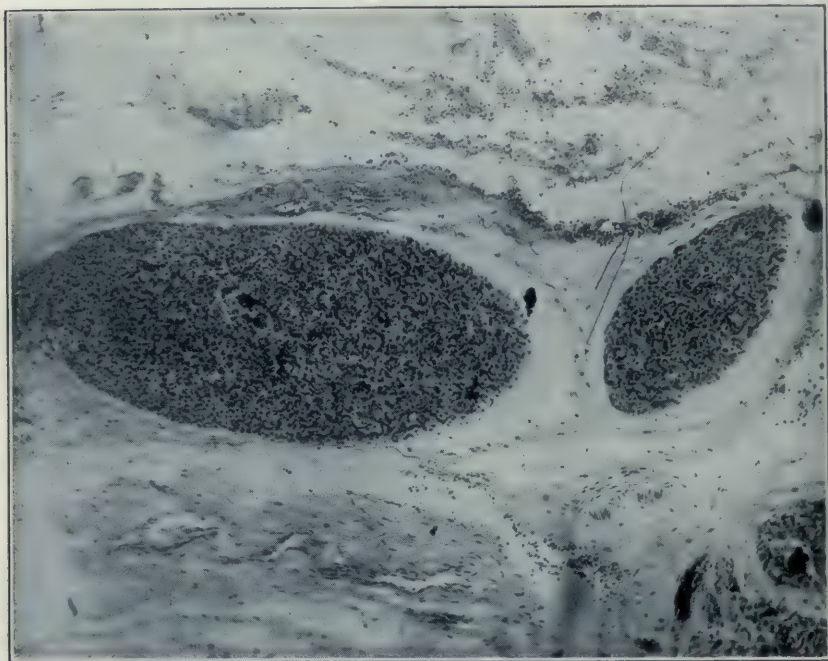


Fig. 1.—Low power photomicrograph showing islands of embryonal fat cells. From a marantic infant. The darker stained areas represent injected capillaries and the lighter stained bodies between them are embryonal fat cells with lightly stained, granular cytoplasm, and moderately chromatic nuclei.

region, but may be seen in practically any locality in the body where fat normally exists. In certain pathologic conditions, however, these collections of primitive fat cells may be increased to such an extent as to resemble a disseminated neoplastic growth, while in the neck they have recently been mistaken for a new variety of gland belonging to the endocrine system (described later).

In the body of an infant investigated post mortem by one of us (Symmers) at the New York Hospital, embryonal fat cells occurred in enormous numbers, and were so distributed as to suggest the pres-

ence of a new growth. The subject was a boy, aged 3 months, dead of inanition. In the loose connective tissues around the larynx, esophagus and thyroid gland, in the fascial planes between the muscles of the anterior and lateral portions of the neck, in the connective tissue between the pectoralis major and minor muscles and the coracobrachialis on the left side, in the loose tissues of the precordium, and in the parietal pleura corresponding to the lower edges of the fourth and fifth ribs on the right side, in the loose tissues immediately behind the sternum from end to end, and behind and in front of both kidneys, especially the left, was a quantity of cherry-red tissue so distributed, practically wherever found, as to form a rough lattice work made up of pea-sized, oval or rounded, glistening bodies arranged discretely or joined by strands of the same general appearance. This trellis-like distribution was particularly noticeable in the retrosternal region, in the precordium, and around the thymus gland, where it was abundant. In the neck, and between the superficial muscles of the thorax, the tissue was found in branching chains. In the connective tissue of the right pleura the tissue formed chains which lay parallel with the edges of the ribs. The tissue in the peritoneum in front of the kidneys, and in the capsules of the kidneys themselves, was arranged in a coarse network. Behind the kidneys the tissue was clumped to form thick, cord-like masses lying in the fascia of the psoas magnus muscles. In specimens fixed in formaldehyd solution the reddish bodies described could be seen breaking up into small angulated masses loosely held together by connective tissue.

Histologic examination showed the presence of innumerable small, rounded, oval, or angulated, richly cellular islands lying in a reticulum of loose connective tissue. The islands were composed of a complex network of injected capillaries, between which were groups of large polyhedral cells, with small, rounded, moderately chromatic, centrally placed nuclei, and finely granular, pinkish cytoplasm. In many of the cells sudan III showed the presence of numerous minute orange-red granules lying in the cytoplasm, while others were free.

In connection with the question of embryonal fat tissues, it is of interest to note that Pende<sup>2</sup> has recently described what he believes to be a new gland of internal secretion belonging to the endocrine system (*glandula insularis cervicalis*). He found it in children and in puppies in the form of from fifteen to twenty solid islands of cells which he assumed to be of epithelial nature and which lay in the connective tissue around the thyroid and thymus glands. The cellular islands appeared as richly vascularized, reddish bodies made up of small,

2. Pende: Arch. f. mikr. Anat., 1915, **86**, 193.



sharply defined cells of rounded or polygonal outline with a centrally placed, vesicular nucleus, and a distinct cell membrane. The cell membrane enclosed numerous small granules so closely packed as to give the cytoplasm a homogeneous appearance, the granules staining pinkish with eosin and responding to special stains for fat. Pende assumed that the granular and fatty substances in the cell body represented the visible expression of an internal secretion. It requires but a glance at the several illustrations which accompany Pende's paper to convince one that the body specifically described by him as a new member of the endocrine series is identical with the primitive fat organs.

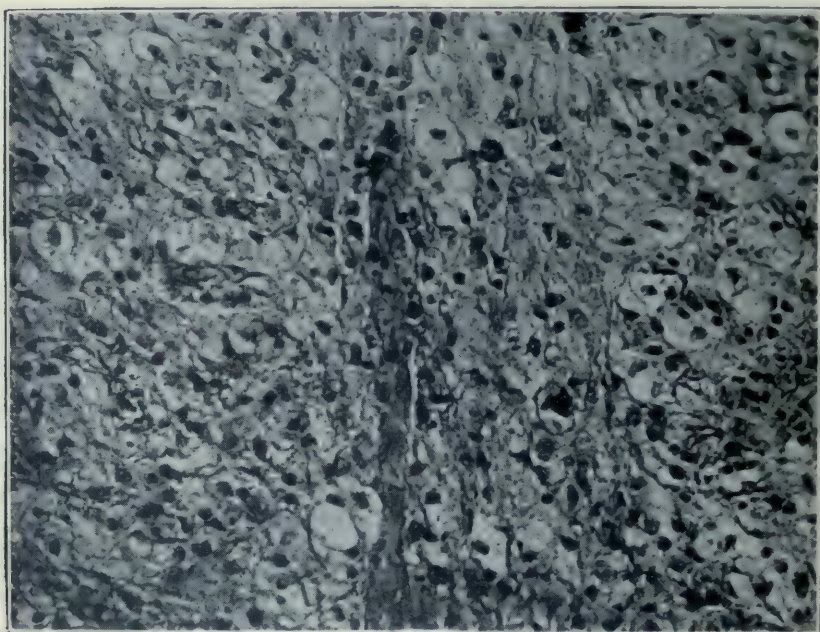


Fig. 2.—High power photomicrograph to illustrate the histologic changes in a disease characterized by interference with the absorption of fat and the deposition of neutral fat and fatty acid crystals in the intestine, mesentery, and mesenteric lymph nodes. Note the enormous hyperplasia of embryonal fat cells. (Nodule from mesentery.)

There is a group of chronic productive inflammatory lesions occurring commonly, but not exclusively, in tissues more or less rich in fat, in which either the pre-existing fat cells undergo proliferation with the production of polygonal forms indistinguishable from the embryonal fat cells, or in which certain numbers of the fibroblastic elements participating in the inflammatory reaction undergo metaplasia into fat cells. We have encountered changes of the sort indicated in the female breast in a case of chronic interstitial mastitis, the fat cells being

arranged in islands lying between masses of dense fibrous tissue. In a chronic productive lesion of the gallbladder and another of the intestine, embryonal fat cells were found widely distributed through the interstitial tissues in the form of narrow strands or as small groups. In a case of chronic interstitial nephritis, the intertubular connective tissues were richly infiltrated, the embryonal fat cells evidently originating in the pelvis and migrating toward the cortex. Like embryonal fat cells elsewhere, they presented an appearance suggestive of infiltrating hypernephroma cells, but the absence of such a growth excluded this possibility. In two chronic productive inflammatory lesions of the mesentery, embryonal fat cells were found diffusely distributed through the connective tissues, and, in a case of tuberculosis of the knee joint, the marrow of the corresponding femur was found to be extensively invaded.

The histologic study of embryonal fat cells in chronic productive inflammatory lesions shows that these cells are migratory. The phenomenon of migration of definitely formed fat cells among alien tissues is obviously different from the infiltration of extracellular fat globules along the line of least resistance, and, in fact, partakes of the nature of an invasive process. It is also exemplified by lipomata composed of embryonal fat cells that display a disposition to invade surrounding tissues, so that a tumor which, in ordinary circumstances, pursues a benign course, is thus diverted in the direction of malignancy. Mallory<sup>3</sup> mentions such a case, and, in the pathologic laboratories at Bellevue Hospital, we have recently encountered a lipoma in the arm of a 16-year-old girl that was composed of embryonal cells in various stages of development. Still another example of the migratory property of embryonal fat cells is to be found in the pathologic histology of the intestine, mesentery and mesenteric lymph nodes in a case recorded by Whipple.<sup>4</sup> The patient was the subject of a disease characterized, clinically, by gradual loss of weight and strength, by the presence of a great abundance of neutral fat and fatty acids in the stools, by vague signs referable to the abdomen, and by a variety of fugitive arthritis involving multiple joints. With the exception of the joints, which could not be investigated post mortem, the anatomic changes were limited to that part of the body mechanism which has to do with the absorption of fats, namely, the small intestine and its lymphatic drainage system. The intestinal villi were enlarged on account of extensive deposits in the lymph spaces of neutral fats and fatty acids, together with infiltration of the interglandular connective

3. Mallory: *Principles of Pathological Histology*, 1914, Ed. 1, p. 207.

4. Whipple: *Bull. Johns Hopkins Hosp.*, 1907, **18**, 382.



tissues by mononuclear cells morphologically identical with embryonal fat cells, and by the presence of multinuclear giant cells. The sub-mucous connective tissues and the mesenteric nodes were similarly affected. The latter structures presented, in addition, microscopic changes which Whipple interpreted as a chronic productive inflammatory process.

As far as we have been able to determine, the case described by Whipple is the only one of the sort in the literature of medicine. What we believe to be a case of the same type, however, recently came under our observation.

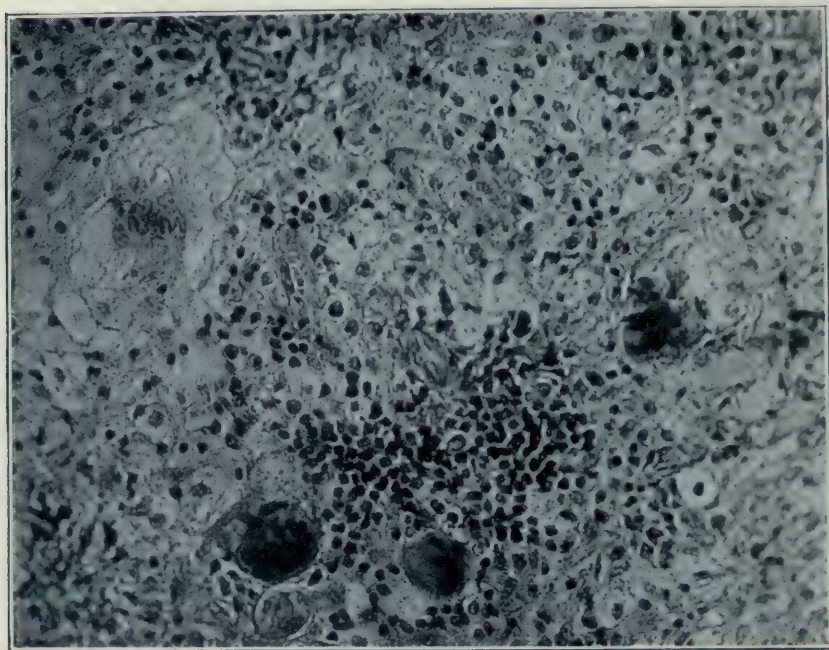


Fig. 3.—Same disease as represented by Figure 2. At the left is a giant cell due to fusion of embryonal fat cells. The other giant cells are of the Langhans type. Note the profusion of embryonal fat cells throughout the field.

The patient, a woman aged 43, was operated on by Dr. George D. Stewart, who found a large, lobulated mass in the region of the pancreas that resembled a group of tuberculous lymph nodes, and exuded a milky fluid on being incised. There were no clinical symptoms referable to disturbances in the absorption of fat, the stools being free. The patient is still under observation, however, and is being watched for the development of manifestations of the sort indicated. The pathologic histology of a small nodule removed from the region of the pancreas at the time of operation, revealed changes in complete agreement with those described by Whipple in the intestine, mesentery, and mesenteric nodes, and illustrated in his paper.

The framework of the nodule was composed of irregularly scattered trabeculae of connective tissue, between which were vast numbers of large, rounded,



oval, or polygonal cells with a distinct limiting membrane, a fairly richly chromatic, centrally placed nucleus, and finely reticulated or granular, pale, poorly staining cytoplasm. In sudan III preparations practically every one of these cells revealed innumerable fat granules in the cytoplasm and overrunning the nucleus. Here and there were a few apparently well developed, fat-free fibroblasts, and an occasional lymphomatous focus. Scattered among the cells were considerable numbers of large fat vacuoles. In many instances the cells in the immediate vicinity of the vacuoles showed no changes beyond slight condensation of their cytoplasm; in other cases the vacuoles were rimmed by multinucleated giant cells, obviously derived from fusion of two or more embryonal fat cells. In occasional instances there were isolated giant cells with an unusually distinct cell membrane; a large amount of pale, finely granular or reticulated cytoplasm, and a half dozen or more well-formed, rounded nuclei, each of which was surrounded by a distinct membrane enclosing, in many instances, a nucleolus. Fat granules were abundant. Mitotic figures could not be found, but evidences of direct nuclear division were visible, and this was apparently the method of nuclear cleavage. The prevailing giant cell, however, was a more or less acceptable counterfeit of the Langhans cell, and was provided with dense, reddish-staining cytoplasm, and multiple, richly chromatic, rounded or oval, well-formed but small nuclei. The cell was practically always built round a fat particle.

Special stains for tubercle bacilli, and Levaditi and Gram preparations were negative.

The histologic changes just enumerated are unique, and occur in no other condition with which we are acquainted. In Whipple's case they were interpreted as a chronic productive inflammatory process — a foreign body granuloma. For our own part, we recognize the probability of the correctness of this view, but are nevertheless impressed by the possibility that the lesion is capable of malignant transformation. In the latter event the growth would constitute a new variety of malignant connective tissue tumor, namely, an embryonal cell liposarcoma or malignant lipoblastoma. The exact nature of the process, however, must remain undetermined until other cases of the same sort have been subjected to detailed investigation.

A phase in the histology of embryonal fat tissues that does not appear to us to have been sufficiently emphasized is to be found in the form of the multinucleated giant cells, which occur in at least three varieties, first, as fusion cells around, or independent of, foreign particles, such as globules of fat; second, as giant cells due to division of the nucleus without corresponding changes in the cell body. This form appears to bear no relationship to the presence of foreign bodies in the tissues, but is best interpreted as an abortive attempt at regeneration; the third variety is a more or less acceptable counterfeit of the Langhans giant cell, and is almost invariably built around particles of fat, cholesterin, or fatty acid crystals, or around stellate bodies of indeterminate nature. The occurrence of stellate inclusions in giant cells was first described by Ris<sup>5</sup> in the walls of an omental cyst. They

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5. Ris: Beitr. z. klin. Chir., 1893, **10**, 423.

have also been detected by Wolbach<sup>6</sup> in the lung, lymph nodes, spleen and liver, by Vogel<sup>7</sup> in a case of obliterating capillary bronchitis, by Iwanzoff<sup>8</sup> in a myomatous uterus, by Mallory<sup>9</sup> in the giant cells of leprosy, and by Wood<sup>10</sup> in a granulomatous lesion of the face following the injection of paraffin for cosmetic purposes. The radiate bodies in question have been variously interpreted as disintegrated elastic tissue, parasitic inclusions, astropheres, fibrinoid bodies, etc. We have recently had occasion to study changes in the fat cells of a regressing corpus

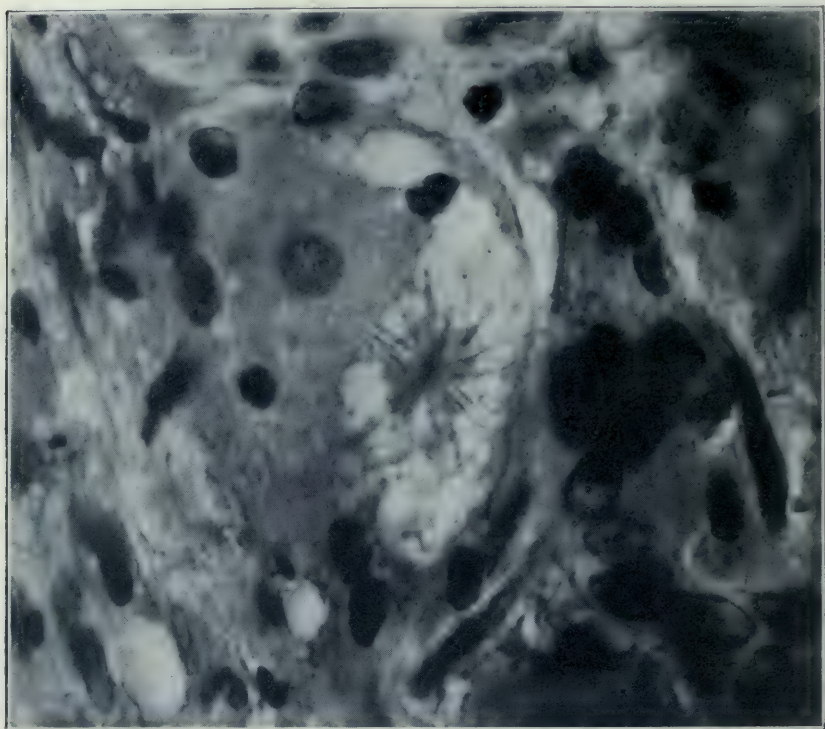


Fig. 4.—Oil immersion photomicrograph showing spiculated body (fatty acid crystal) in a giant cell of a regressing corpus luteum.

luteum attended by the formation of enormous numbers of giant cells, some of which were arranged around cholesterolin crystals, others enclosing a single star-shaped body in the cytoplasm. The shape of these inclusions, and their occurrence in degenerate cells rich in fat, together with the presence in neighboring giant cells of lipoids in the

6. Wolbach: *Jour. Med. Research*, New Series, 1911, **19**, 243.

7. Vogel: *Virchows Arch. f. path. Anat.*, 1911, **206**, 157.

8. Iwanzoff: *Beitr. z. path. Anat.*, 1912, **52**, 202.

9. Mallory: *Loc. cit.*, Note 3, p. 303.

10. Wood: *Proc. New York Path. Soc.*, New Series, 1915, No. 5, p. 63.

form of cholesterin, incline us to the view that the stellate bodies in the regressing corpus luteum bear a definite relationship to the disintegration of fats, and are most probably of the nature of fatty acid crystals.

#### CONCLUSIONS

The study of embryonal fat cells in certain pathologic conditions suggests the following conclusions:

1. The histogenesis of fat cells is brought about in two ways; first, in the form of connective tissue cells set apart in embryonal life for the specific purpose of producing fat, and, second, as the result of metaplasia of fibroblasts with the accumulation of fat in the cytoplasm and the assumption by the cell of the polygonal form of the embryonal unit.

2. There is a condition in marantic infants attended by such extensive hyperplasia of the so-called primitive fat organs as to resemble a new growth. The fat bodies are cherry-red in color and are composed of embryonal fat cells and a complex system of intercommunicating capillaries. The bodies may be found wherever fat tissues normally exist, but are particularly numerous in the connective tissue planes of the neck and chest and around the adrenals and kidneys.

3. The *glandula insularis cervicalis* of Pende, recently described as a new member of the endocrine series, is identical in structure and function with the embryonal fat organs.

4. There is a group of chronic productive inflammatory lesions and a variety of lipoma attended by marked hyperplasia of embryonal fat cells in which these cells not infrequently display a distinct disposition to migrate into alien tissues. Moreover, there is a disease of the fat absorption apparatus characterized, histologically, by neutral fats and fatty acids in the lymph spaces of the intestine, mesentery and mesenteric lymph nodes, the tissues showing, in addition, extensive hyperplasia of embryonal fat cells and the presence of foreign body giant cells. (Whipple, Symmers and Fraser.) The histology is indicative of a chronic productive inflammatory process attended by such extensive hyperplasia of embryonal fat cells as to suggest transformation into a sarcoma (embryonal cell liposarcoma, malignant lipoblastoma).

5. Embryonal fat cells are phagocytic, and may form multinucleated giant cells around fat globules, cholesterin crystals, and the like. In other circumstances the cell, in attempting to divide, forms giant cells by repeated cleavage of the nucleus without corresponding changes in the cell body. Giant forms resulting from fusion of embryonal fat



cells also occur. In all of these varieties the cytoplasm reveals fat granules in abundance.

6. The giant cells occasionally encountered in the tissues of the regressing corpus luteum are built round foreign bodies in the form of cholesterin crystals, and the stellate radiations sometimes to be observed in the cytoplasm are probably fatty acid crystals due to disintegration of the fat cells of the part.

We are indebted to Dr. John E. McWhorter for the photomicrographs.  
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## EXPERIMENTAL APPENDICITIS \*

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Although appendicitis has long been definitely recognized as a clinical entity, there is at the present time no unity of opinion among the various investigators as to its pathogenesis. Most authors agree, however, that infection is an important factor in its production. One finds a variety of predisposing factors described as favoring the infection, but there is a disagreement as to the mode of entrance of the offending organisms into the tissue of the appendix.

Aschoff<sup>1</sup> has maintained that the disease has an enterogenous origin, while Kretz<sup>2</sup> has endeavored to demonstrate the localization of organisms in the follicular apparatus of the appendix by way of the blood stream, gaining entrance thereto, particularly, from the tonsils. The latter investigator was of the opinion that the streptococcus is the exciting cause, as he could demonstrate gram-positive cocci within the vessels in the submucosal follicles. Recently Rosenow,<sup>3</sup> who has studied the question of hematogenous origin of appendicitis, has endeavored to prove that streptococci isolated from diseased appendixes, or from the tonsils of patients suffering from appendicitis, possess certain transient, biologic characteristics which favor invasion of the appendix. This affinity he designates "organotropism." He asserts that organotropism is not possessed by organisms isolated from sources other than the disease focus or apparent atrium of infection. In order that this affinity may be preserved, it is requisite that the bacteria shall be isolated and handled in accordance with a particular bacteriologic technic. This affinity is transient and cannot be preserved through more than a limited number of generations, either on artificial cultivation or by animal passage. Rosenow also found that such an organotropism is not confined to the bacteriology of the appendix, but exists for the stomach and duodenum in ulcer, for the gallbladder in cholecystitis and for the joints in arthritis. There is no doubt that organisms on gaining entrance to human or animal bodies grow most luxuriantly in that tissue which affords most suitable environment. In this connection one, however, cannot overlook the possibility of sensitized or particularly susceptible tissues rather than variations in the organisms. In 1901 Adrian<sup>4</sup> injected various organisms intravenously into rabbits and found the appendix so regularly affected that he said this organ in rabbits is a place of predilection for the localization of organisms circulating in the blood. The relation of the tonsils to certain types of infection is apparently

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\* From Cornell University, Surgical Division, and the Pathological Department of Bellevue Hospital.

1. Aschoff: Verhandl. d. deutsch. path. Gesellsch., 1904, **7**, 246; *ibid.*, 1906, **10**, 234; *ibid.*, 1907, **11**, 313. Die Würmfortsatzentzündungen, Jena, 1908.

2. Kretz: Verhandl. d. deutsch. path. Gesellsch., 1906, **10**, 229; *ibid.*, 1907, **11**, 309; *ibid.*, 1910, **14**, 144; Ztschr. f. Heilk., 1907, **28**, 151; Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1907, **17**, 1.

3. Rosenow: Jour. Infect. Dis., 1914, **14**, 61; *ibid.*, 1915, **17**, 240; *ibid.*, 1916, **18**, 383.

4. Adrian: Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1901, **7**, 407.

well established at the present time. It is not conceivable, however, that these organs have such a varied property that they are able to impart peculiar characters to organisms whereby an individual bacterium may possess different affinities at several distinct periods.

In view of the widespread lesions observed in human bacteremia, and with a desire to obtain more direct information on this subject, we decided to study the effects on rabbits of the intravenous injection of micro-organisms derived from several sources. At this time we wish to describe at length the appendiceal lesions observed, reserving the general description of the bacteremia produced for a later communication.

Material for this work was obtained from appendixes, from the tonsils of patients having appendicitis, from several pairs of tonsils and adenoid tissue removed in the children's clinic, from tonsils of fracture patients otherwise apparently healthy, and from pus of an infected hand. Among the appendixes there were included two normal appendixes, two acute ulcerative appendixes, three acute gangrenous appendixes (two with concretion and perforation), three chronic appendixes, peritoneal fluid in one case of appendicitis, and pus from an abscess in the abdominal incision following operation for acute gangrenous appendicitis.

#### TECHNIC

All cultures from the appendixes were made from the mucosal surface after opening them longitudinally under sterile precautions. Where the mucosa presented macroscopical lesions the organ was first washed in running sterile salt solution, after which material was scraped from the base of the diseased area. In the case of the perforated organs, cultures were made from the borders of the opening. The normal appendixes were ground up in a sterile mortar with sterile white sand after the organs were thoroughly washed. The tonsils studied consisted of those from five of the patients having appendicitis and those of thirteen patients not suffering with this disease; of the latter group, ten were from patients with fractures but otherwise well, while the remaining three consisted of tonsils removed from children affected with chronic tonsillitis. A mass of adenoid tissue from one of the latter three was also used for culture. Where the tonsils were removed, and likewise in the case of the adenoids, the entire tissue was ground up in a sterile mortar with sterile white sand. Cultures from the tonsils of the appendix patients, and also from the fracture patients, were made by boring sterile swabs into the crypts of the organs. The material was in each case inoculated into 150 c.c. of dextrose serum broth. The serum broth was prepared according to the method described by Holman.<sup>5</sup> During the first part of the work 1 per cent. of dextrose was used, while this was later changed to two-tenths per cent.; the only appreciable difference seemed to be that more luxuriant growths occurred in the serum broth containing the stronger percentage of the carbohydrate. These cultures were incubated at 37 C. for twenty-four hours, when the flask was vigorously shaken, the fluid placed in sterile 50 c.c. tubes and centrifugalized at 3,000 revolutions for about fifteen minutes, until the supernatant fluid was perfectly clear. The sediment was then collected and suspended in 10 c.c. of sterile salt solution, whereby each cubic centimeter of the suspended sediment represented 15 c.c. of the original growth, except when the

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5. Holman: *Jour. Infect. Dis.*, 1914, **15**, 209, 293.



entire sediment was injected into the ear vein in one dose, in which case it was taken up in 5 c.c. of saline. The majority of the animals received doses varying from 15 to 75 c.c. of the original growth. Before injection plain human blood agar streak plates were made and the nature of the organisms in the emulsion determined.

The relation between dosage and effect was at times inconstant. Rabbits injected with equal doses of the same suspension would show one animal living and the other dead at the end of twenty-four hours.

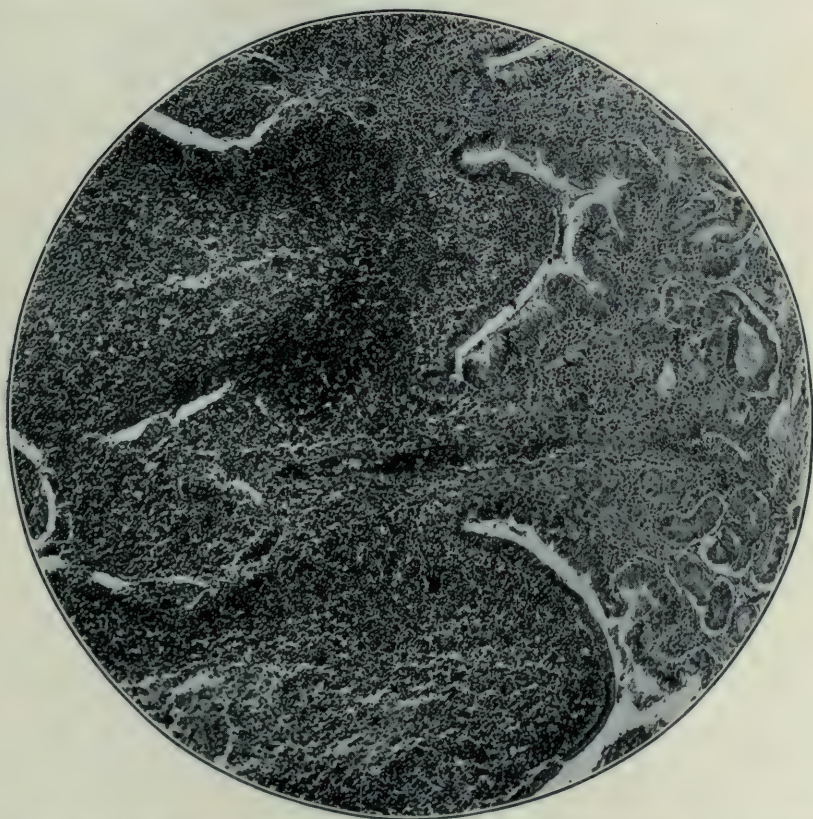


Fig. 1.—Appendix, Rabbit 75, showing marked hemorrhage into and between the follicles.

Again, on several occasions, an animal receiving a dose of 75 c.c. survived, while two receiving 30 c.c. and 45 c.c., respectively, died. From this there appeared to be an individual variation in resistance among the rabbits, so that in some of the animals which received a smaller dose there was developed a severe bacteremia with many lesions, while another rabbit, inoculated with a larger dose of the same material, when killed revealed but few alterations in its organs. Although this event was not the rule, these facts were definitely observed and should

be considered as very important when interpreting the results obtained in animal experimentation. This is a matter worthy of the serious attention of all workers in this field.

#### TONSILS IN APPENDIX CASES (TABLE 1)

Some of the experiments consisted of the inoculation into the ear vein of the mixed culture, the nature of the organisms given being

TABLE 1.—DATA CONCERNING TH

Case	Source	Organisms	Rabbit No.	Rab- bits In- jected	Ap- pen- dix	Skin	Lung	Stom- ach	Heart	Thi
68	Tonsil (chronic appendicitis) (Case 67)	Staph. aureus; B. xerosis	56	1	1	1	1	...	1	
69	Tonsil (acute gangrenous appendicitis) (Case 69)	Staph. aureus; Staph. albus	61	1	1	...	1	1	1	
		S. pyogenes-vulgaris (append., Rabbit 61)	74	1						
71	Tonsil (acute ulcerative appendicitis) (Case 70)  See Case 71 (Table 2) for detail	S. salivarius; Staph. albus	64	1	1	1	1	1	1	
		S. salivartus (knee, Rabbit 64) into lumen	78	1	1					
		S. salivarius (into appendix artery)	79, 83, 84, 85	4	4	1	3	3	2	
		S. salivarius.....	80, 90, 91, 92	4	...	3	2	1	2	
		S. salivarius.....	43, 59, 82, 88, 104, 105	6	2	3	2	3	2	
		S. salivarius (peritoneum, Rabbit 83)	119, 120, 121	3	1	3	...	3	...	
		S. salivarius (periarticular, Rabbit 43)	122, 123, 124	3	1	3	3	2	1	
73	Tonsil (chronic appendicitis) (Case 72)	S. mitis; Staph. albus	68	1	...	...	1	...	...	
		S. subacidus; Staph. albus	130, 131, 132, 133	4	2	3	2	2	1	
87	Tonsil.....	S. pyogenes-vulgaris (knee, Rabbit 130)	160, 161	2	1					
5	Totals.....			35	16	21	16	16	12	

determined by previous plating. On the other hand, some organisms, particularly streptococci, were carried through several animal passages and showed interesting deviations in the frequency with which the different organs were attacked. To determine whether or not the repeated cultivation of an organism on artificial mediums or its passage through animals had any influence on the power of attacking the

appendix, we selected material from the tonsils of a patient suffering with acute ulcerative appendicitis and cultured it in the manner described. The streptococcus isolated from this case, B-71-16, produced slightly raised, glassy colonies with distinct greening on plain blood agar, which later exhibited some browning. To determine its power of ferment production, the organism was tested against lactose, mannite, salicin and inulin in serum broth as described by Holman,

## TONSILS IN APPENDIX CASES

no.- e- um	Joint	Small Intes- tine	Peri- articu- lar	Brain	Endo- car- ditis	Kid- ney	Cord	Cecum	Peri- car- dium	Mus- cle	Pleura	Gall Blad- der	Liver	Large Bowel	Eye
1	...	1	...	...	...	1	...	...	1						
...	...	1													
1	1	...	...	...	1	1	...	...	1	...	1				
...	1	2	...	...	...	...	...	2	...	...	1	1			
...	...	...	...	...	...	1	...	...	...	...	...	1			
2	2	1	2	...	1	1	...	1	1	2	...	...	2	1	
2	...	...	1	2	1	...	1	...	...	1	...	...	...	...	1
2	2	...	1	...	1	...	1								
2	...	...	...	3	...	...	2								
...	...	1													
1	4	...	1												
1	10	6	5	5	4	4	4	3	3	3	2	2	2	1	1

and was found to attack only lactose, the observation being continued for fourteen days. Morphologically, the organism was seen in hanging drop preparation from the lactose serum broth to occur in pairs and pairs in chains of ten and more round or slightly elongated cocci. The organism was gram-positive. It conformed, therefore, in all particulars to the *Streptococcus salivarius*. All of the streptococci for this work



were studied in this manner at all stages of the different experiments. Following the isolation of this streptococcus from the original tonsil (Case B-71-16) and after animal passage, the organisms were cultured on plain 5 per cent. human blood agar, transfers being made to new blood agar slants each time a subculture was made. The sediment of the original growth was suspended in 5 c.c. of normal saline solution and inoculated into the ear vein of Rabbit 64. The animal died two days later and at necropsy the appendix exhibited numerous pinpoint

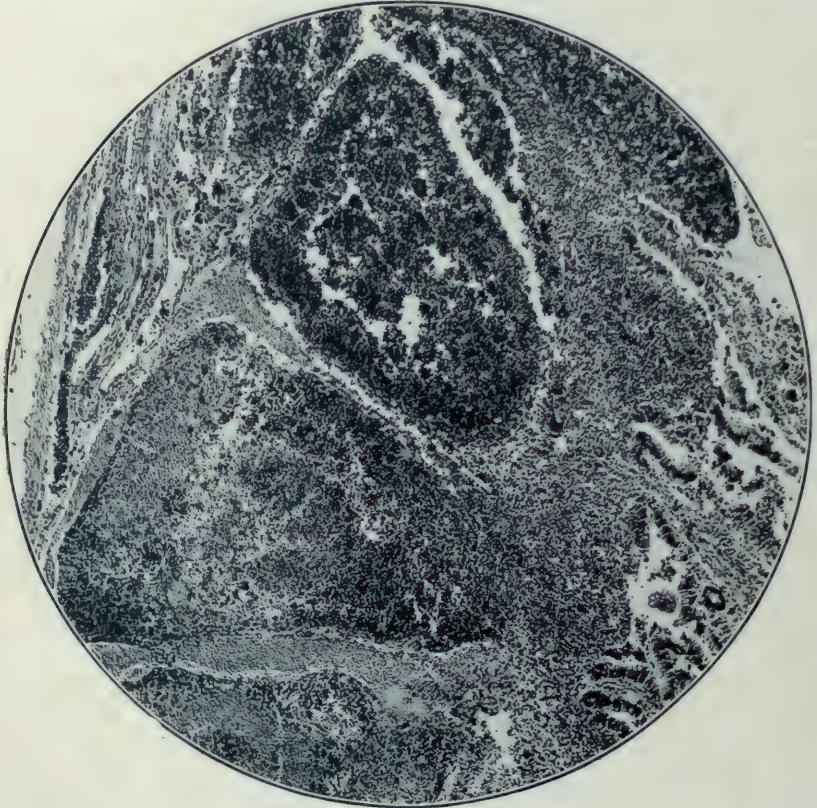


Fig. 2.—Appendix, Rabbit 84, showing two follicles almost completely replaced by hemorrhage, which also extends to the surface of the mucosa.

red areas in the mucosa of its middle portion together with hemorrhages into the skin, endocardium, lung, stomach, duodenum, pleura and pericardium, with a vegetative endocarditis involving the tricuspid valve and lesions observed as small white dots in the heart muscle and kidney. From the heart blood and knee joints a *S. salivarius* was isolated. The material with which Rabbit 64 was injected came to the laboratory January 16, and on culture showed a *S. salivarius* and *Staphylococcus albus*.

On January 28 two rabbits, 78 and 79, inoculated with small doses of the *S. salivarius* respectively into the lumen of the appendix and artery of the organ, presented only local reaction, while Rabbit 80 received the growth from 150 c.c. into the ear vein, and when killed six days later showed no alterations in its organs. Three rabbits, 83, 84 and 85, injected January 31 into the appendiceal artery with small doses, not only presented a local appendicitis, but also lesions in other organs, thus demonstrating that the streptococcus, although given every opportunity, did not tend to produce lesions solely in the appendix. A *S. salivarius* isolated from the knee joint of Rabbit 83 and carried along on plain blood agar until February 15, was again grown in 150 c.c. of dextrose serum broth and inoculated in divided doses into the ear veins of Rabbits 43, 59 and 82. Two of these animals, 43 and 59, which received, respectively, 30 and 45 c.c., died, and at necropsy showed lesions in the appendix and many other organs, while Rabbit 82, which received 75 c.c., lived, and when killed two days later showed lesions only in the stomach and lung. Again, on February 19, three rabbits (119, 120 and 121) injected, respectively, with 40, 60 and 50 c.c. of a *S. salivarius* from the peritoneum of Rabbit 83, showed the appendix affected only in Rabbit 120. Three rabbits (122, 123 and 124) received, respectively, 30, 45 and 75 c.c. of a *S. salivarius*, isolated from a hemorrhage about the knee joints of Rabbit 43, and showed the appendix affected in but one animal, Rabbit 123. February 24, three rabbits (125, 126 and 127) inoculated, respectively, with 30, 45 and 75 c.c. of a *S. salivarius* from the blood of Rabbit 43, revealed the appendix affected in one only, rabbit 127. The details of these experiments are presented in Table 2, Case 71.

From the results described it will be noted that the rabbits' appendixes were affected in each of four groups with a *S. salivarius* derived from a source other than the appendix; that is, from the knee joint, the periarticular tissues, the peritoneum or the heart blood, and that the animal receiving the largest dose in any group was not necessarily the one to exhibit changes in its appendix. Lesions in the appendix followed the intravenous injection of the streptococcus from each of the sources named. Another interesting feature displayed by this streptococcus during the time it was studied consisted in the marked variation with which it attacked the different organs; even in animals injected with doses from the same subculture, no definite order of invasion was followed. That the organism apparently did not tend to lose its power of attacking the appendixes of some rabbits is demonstrated by the production of an appendiceal condition in five of twelve rabbits thirty days after the original isolation (B-71-16), only one animal passage having supervened. From the time of the first animal passage when the organism was isolated from the knee joint of Rabbit

TABLE 2.—DETAILED DATA—

No.	Date	Source	Material	Amt. C.c.	Method	Death	Date	Skin	Heart	Lung	Stom- ach	Duo- de- num	Ap- pen- dix
64	1/17	Tonsil B-71-16	Mixed	150	Ear vein	Died	1/19	+	+	+	+	+	+
78	1/28	Knee joint R-64-16	S. salivarius	½	Lumen of appendix	Killed	1/30	—	—	—	—	—	+ Local
79	1/28	B-71-16	S. salivarius	1	Appendix artery	Killed	1/30	—	—	—	—	—	+ Local
80	1/28	B-71-16	S. salivarius	150	Ear vein	Killed	2/ 3	—	—	—	—	—	—
83	1/31	B-71-16	S. salivarius	½	Appendix artery	Died	2/ 2	—	+	+	+	—	+ Local
84	1/31	B-71-16	S. salivarius	1	Appendix artery	Died	2/ 1	—	—	+	+	—	+ Local
85	1/31	B-71-16	S. salivarius	1	Appendix artery	Died	2/ 1	+	+	+	+	—	+ Local
90	2/ 2	B-71-16	S. salivarius	150	Ear vein	Died	2/ 3	+	+	+	—	—	—
91	2/ 4 2/ 5	B-71-16	S. salivarius	10-10	Ear vein	Killed	2/ 7	+	—	—	—	—	—
92	2/ 4 2/ 5	B-71-16	S. salivarius	10-10	Ear vein	Died	2/ 7	+	+	+	+	—	—
104	2/14	Knee joint R-83	S. salivarius	30	Ear vein	Killed	2/15	—	+	—	+	—	—
105	2/14	Knee joint R-83	S. salivarius	75	Ear vein	Killed	2/15	+	—	—	—	—	—
88	2/14	Knee joint R-83	S. salivarius	45	Ear vein	Killed	2/15	—	—	—	—	—	—
43	2/16	Knee joint R-83	S. salivarius	30	Ear vein	Died	2/17	+	+	+	+	+	+
59	2/16	Knee joint R-83	S. salivarius	45	Ear vein	Died	2/17	+	—	—	—	+	+
82	2/16	Knee joint R-83	S. salivarius	75	Ear vein	Killed	2/18	—	—	+	+	—	—
119	2/19	Peritoneum R-83	S. salivarius	40	Ear vein	Killed	2/22	+ Also lip	—	—	+	+	—
120	2/19	Peritoneum R-83	S. salivarius	60	Ear vein	Killed	2/21	+	—	—	+	+	+
121	2/19	Peritoneum R-83	S. salivarius	50	Ear vein	Killed	2/22	+ Lip	—	—	+	—	—
122	2/22	Skin knee R-43	S. salivarius	30	Ear vein	Killed	2/23	+	—	+	—	+	—
123	2/22	Skin knee R-43	S. salivarius	45	Ear vein	Died	2/23	+	+	+	+	+	+
124	2/22	Skin knee R-43	S. salivarius	75	Ear vein	Died	2/23	+	—	+	+	—	—
125	2/24	Blood R-43	S. salivarius	30	Ear vein	Killed	2/25	+	+	—	—	+	—
126	2/24	Blood R-43	S. salivarius	45	Ear vein	Killed	2/28	+	—	—	—	—	—
127	2/24	Blood R-43	S. salivarius	75	Ear vein	Died	2/27	+	—	—	—	+	++
25	.....	.....	.....	.....	.....	.....	.....	17	9	11	13	9	11



## —CONCERNING CASE 71

Small Intes-tine	Cecum	Large Bowel	Eye	Pleura	Gland	Mus-cle	Endo-car-ditis	Peri-car-dium	Kid-ney	Gall-blad-der	Liver	Joint	Peri-articu-lar	Brain	Cord
—	—	—	—	+	—	—	+	+	+	—	—	+	—	..	..
—	—	—	—	—	—	—	—	—	—	—	—	—	—	..	..
—	—	—	—	—	—	—	—	—	—	—	—	—	—	..	..
—	—	—	—	—	—	—	—	—	—	—	—	—	—	..	..
—	+	—	—	+	—	—	—	—	—	+	—	+	—	..	..
+	+	—	—	—	—	—	—	—	—	—	—	—	—	..	..
+	—	—	—	—	+	—	—	—	—	—	—	—	—	..	..
—	—	—	—	—	—	—	—	—	+	+	—	—	—	..	..
—	—	—	—	—	—	—	—	—	—	—	—	—	—	..	..
—	—	—	—	—	—	—	—	—	—	—	—	—	—	..	..
—	—	—	—	—	—	—	—	—	—	—	+	—	—	..	..
—	—	—	—	—	—	—	—	—	—	—	—	—	—	..	..
—	—	—	—	—	—	—	—	—	—	—	+	—	—	..	..
—	—	+	—	—	+	+	—	—	—	—	—	+	+	..	..
+	+	—	—	—	+	+	+ TO	+	+	—	—	+	+	..	..
—	—	—	—	—	—	—	—	—	—	—	—	—	—	..	..
—	—	—	—	—	—	—	+ MO	—	—	—	—	—	—	+	+
—	—	—	—	—	+	+	—	—	—	—	—	—	+	—	—
—	—	—	+	—	—	—	—	—	—	—	—	—	—	+	—
—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	+
—	—	—	—	—	—	—	—	—	—	—	—	+	+	—	—
—	—	—	—	—	+	—	+ TO	—	—	—	—	—	—	—	—
—	—	—	—	—	+	—	—	—	—	—	—	—	—	+	+
—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+
3	3	1	1	2	7	3	4	2	3	2	2	6	4	5	4

83, it was cultured for the next sixteen days on plain blood agar, and when the growth from 150 c.c. of a subculture in dextrose serum broth was inoculated into Rabbits 43, 59 and 82, the appendix was affected in two instances, Rabbits 43 and 59. Although the organism had been tried in six animals during the preceding sixteen days, the appendixes in all were unaffected. This lapse in its ability to affect the appendix

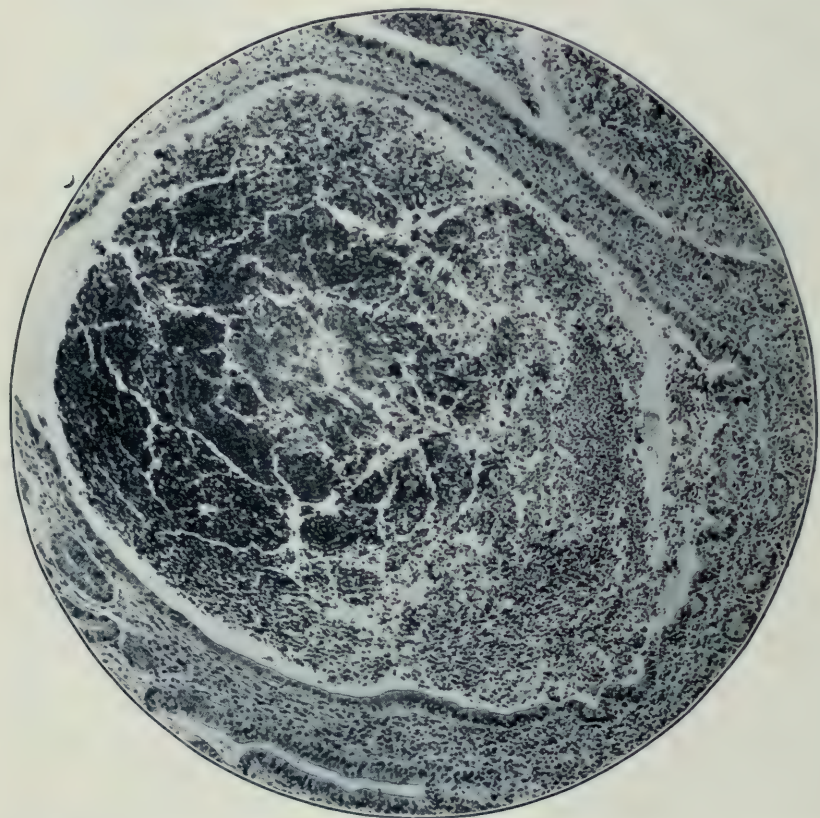


Fig. 3.—Appendix, Rabbit 106, showing a large clump of necrotic material, with leukocytes and red blood cells in the lumen of a gland, with invasion of the mucosa in several places. There are many eosinophils, with a considerable number of leukocytes in the adjacent submucosa.

is a matter difficult of explanation; however, it certainly cannot be attributed to the effect of a varying oxygen tension, to which the organism might have been subjected, as suggested by Rosenow, since it was constantly maintained on plain blood agar slants in cotton-stoppered tubes. This variation in the tendency to attack the appendix at different times is one which should receive the most careful consideration in the interpretation of experiments, since one would be

very prone to error if the organism was not studied over a sufficient period.

It was found that the streptococcus still retained its ability to attack the appendixes of some rabbits when inoculations (125, 126 and 127) were made thirty-eight days after the original isolation, hemorrhages occurring in the appendix of Rabbit 127. As appendiceal lesions occurred in five of twelve rabbits injected during the last eight days, as against no appendicitis in six rabbits in the previous sixteen days, it would appear that the animals themselves must possess peculiar tissue variations, thus limiting the precision with which the biologic characters of an organism can be determined by its effect on living animal tissue. This observation is one of first importance. In any event, the results obtained by injecting the twenty-five rabbits of this group with successive generations of a *S. salivarius* from a parent strain derived from the tonsil of a patient in the height of an attack of acute appendicitis, have demonstrated that the invasive quality of this bacterium, a recognized member of the *S. viridans* group commonly found in the mouth, is a most variable quantity. Moreover, we are not inclined to believe that these peculiarities can be attributed to special methods, as we have at no time followed technic other than that described, which in itself is but one of the authoritative present-day bacteriologic practices.

Material from the tonsils of four other appendicitis patients cultured in the manner described and injected into ten rabbits, produced lesions in the appendixes of five of these animals. Three of the ten rabbits (56, 61 and 68) received the sediment from the entire growth, Rabbits 56 and 61 showing their appendixes involved. A streptococcus (*S. mitis*) was isolated from the original material in but one instance, and the rabbit (68) receiving this mixture showed no change in its appendix. However, a streptococcus *pyogenes vulgaris* was recovered from the appendix of Rabbit 61, and when inoculated in full amount into the ear vein of Rabbit 74, the animal's appendix was unchanged. As there were no other lesions found in this animal's body, it is probable that the culture was sterile. The animal died four days later with the cultures at necropsy negative (heart blood, bile, knee joint). Thus with a total of thirty-five rabbits injected with organisms obtained from the tonsils of appendix patients, sixteen developed lesions in their appendixes. Four of the above sixteen were inoculated locally into the appendix artery, and in one case into the lumen of the organ, so that there were but eleven animals treated intravenously which exhibited appendicitis. It was worthy of note, however, that where the organisms were given into the appendiceal artery there was little tendency toward localization exclusively in the appendix tissues. Even in such an experiment where the organisms were afforded every opportunity of



localizing only in the appendix, it was found that they filtered through the tissues of this organ, and once having gained entrance to the general circulation, the picture of acute bacteremia was developed.

# MATERIAL FROM APPENDIXES (TABLE 3)

The appendixes from which material was used have been divided into groups comprising normal organs, acute ulcerative, acute gangren-

TABLE 3.—RESULTS OF INOCULATIONS—

Case	Source	Organisms	Rabbit No.	Rabbits In-jected	Ap-pen-dix	Stom-ach	Skin	Lung
9	Normal appendix....	<i>S. mitis</i> ; <i>B. coli-communis</i> ; Influenza-like <i>B.</i>	36	1	...	1	...	...
49	Acute ulcerative ap-pendicitis	<i>S. fecalis</i> .....	39, 73	2	...	2	...	1
		<i>S. fecalis</i> ; <i>B. coli-communior</i> ; <i>Staph. albus</i>	41	1				
67	Chronic appendicitis	<i>B. acidi-lactici</i> ; <i>Staph. albus</i>	55	1	1	1	1	1
		<i>B. acidi-lactici</i> .....	63, 93, 94	3	2	3	3	3
		<i>Staph. albus</i> .....	65	1	...	1	...	...
		<i>Pneumococcus</i> (R 55).....	89	1	...	1	...	1
69	Acute gangrenous appendicitis	<i>B. acidi-lactici</i> .....	60, 66, 76	3	2	2	3	2
		<i>B. acidi-lactici</i> (R. 76).....	86	1	1	1	...	1
		<i>Staph. albus</i> (R. 73 and 76)	87	1	1	...	...	...
70	Acute ulcerative ap-pendicitis	<i>B. coli-communior</i> .....	62	1	...	1	1	1
72	Chronic appendicitis	<i>B. coli-communis</i> ; <i>B. xerosis</i>	67	1	1	1	1	1
74	Chronic appendicitis	<i>B. coli-communis</i> .....	69	1	...	1	...	...
75	Acute gangrenous appendicitis (perforation)	<i>B. acidi-lactici</i> .....	70	1	1	1	1	1
76	Pelvic fluid (Case 75)	<i>Staph. albus</i> ; <i>B. Friedlander</i> ; <i>B. xerosis</i>	71	1	1	1	1	...
78	Acute gangrenous appendicitis	<i>B. acidi-lactici</i> .....	72	1	1	1	1	1
82	Normal appendix....	<i>B. coli-communis</i> .....	101, 102, 103	3	1	3	3	3
		<i>S. equinus</i> (R. 101).....	110, 111, 112, 116, 117, 118	6	2	4	4	5
83	Abscess, abdominal incision (Case 69)	<i>S. subacidus</i> .....	81, 106, 107, 108, 113, 114	6	...	3	4	1
12 cases in all. ....				36	14	28	23	22

ous and chronic appendicitis. Ten rabbits were inoculated with the material obtained from two normal appendixes, only three of which developed lesions in their appendixes. One animal (36) injected with a mixture of *Streptococcus mitis*, *B. coli-communis* and an influenza-

like organism from one of these appendixes exhibited no change in its appendix, while three rabbits (101, 102 and 103) which received a *B. coli-communis* isolated from another normal appendix, showed one (103) with its appendix affected. A *S. equinus* recovered from the gallbladder of Rabbit 101 was subsequently inoculated into six rabbits (110, 111, 112, 116, 117, 118), two of which animals had their appendixes affected.

## —WITH MATERIAL FROM APPENDIXES

Heart	Duo- denum	Small Intes- tine	Joint	Thy- mus	Cecum	Eye	Peri- car- dium	Pleura	Gall- blad- der	Mus- cle	Large Bowel	Peri- articu- lar	Liver	Endo- car- ditis	Kid- ney
1	...	...	...	...	...	...	...	...	...	...	...	...	...	1	
1	2	...	...	1	...	1	1	...	...	...	...	...	1		
1	1	1	1	...	1	1	...	...	...	...	1	...	1	...	1
2	1	2	2	...	2	1	...	...	...	1	1				
...	...	...	...	...	...	...	...	...	1						
1	...	...	...	1	...	...	...	...	...	...	...	1			
2	3	3	2	2	...	1	1	1	3	2	1				
1	...	1	1	1	...	...	...	...	1						
...	1	1	...	...	1										
1	1	1	1	1	1	1	...	...	...	...	...	1	...	1	
1	1	1	1	1	1	1	1	1	...	1	...	...	...	1	1
1															
1	1	1	1	...	1	1	...	...	1						
1	1	1	...	...	1	1	...	...	...	...	1				
1	1	1	1	1	1	...	...	...	...	...	1	...	1	...	1
2	2	1	3	3	...	1	3	3	1	...	...	...	1		
2	3	...	...	1	2	...	...	1	...	2	...	1			
2	1	1	...	...	...	...	1	1	...	...	...	1			
21	19	15	13	12	10	10	7	7	7	6	5	4	4	3	3

Of the animals injected with material from cases of acute ulcerative appendicitis, none had the appendix involved. Three animals (39, 41 and 73) received cultures of organisms isolated from one case. The first, 41, was given the mixture of isolated organisms including *S.*

*fecalis*, *B. coli communis* and *Staphylococcus albus*, while 39 and 73 received pure cultures of *S. fecalis*. The appendixes of all of these animals were unaffected. Likewise another rabbit (62), inoculated with a *B. coli-communis* from a second case of acute ulcerative appendicitis, escaped with its appendix unchanged.

Three cases of acute gangrenous appendicitis yielded pure cultures of *B. acidi-lactici*. Three animals (60, 66 and 76) injected with the material from one case presented two diseased appendixes (60, 66).

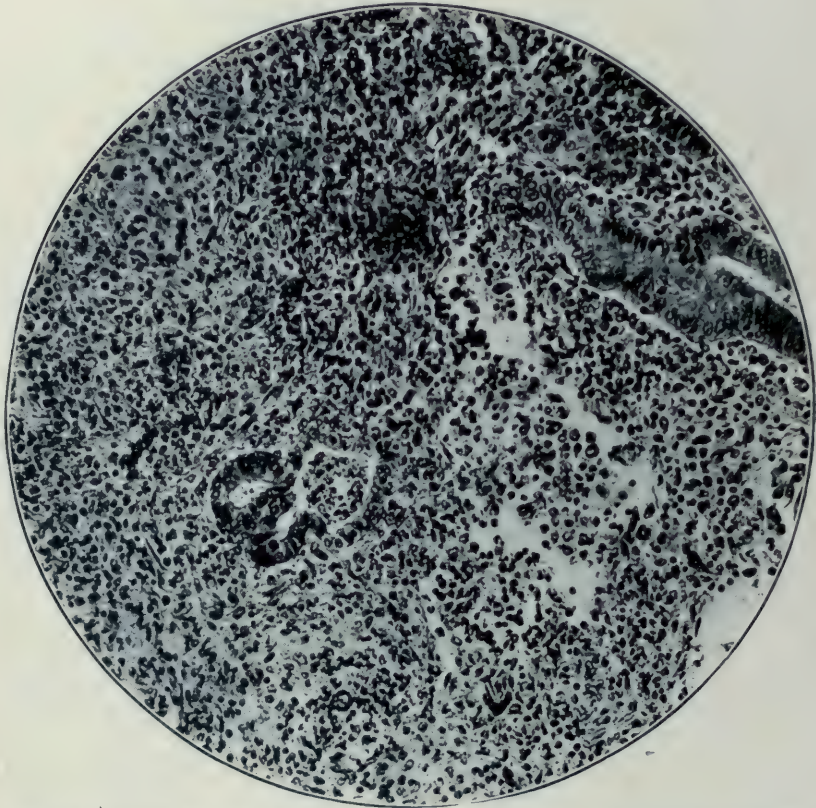


Fig. 4.—Appendix, Rabbit 80, showing an infiltration of leukocytes, lymphocytes and endothelial cells in the submucosa. A clump of debris with leukocytes is observed in the lumen of the gland.

Two other animals (70 and 72) inoculated, respectively, with the material from the other two cases, showed alterations in the appendix. A rabbit injected with the mixed culture (76)—(*B. Friedländer*, *Staphylococcus albus*, *B. xerosis*)—from the pelvic fluid from Case 75, had an affected appendix at necropsy. Six animals inoculated with a *S. ignavus* (83), obtained from the infected wound of Case 69 showed no alteration in their appendixes. Another animal treated with a



*B. acidi-lactici* (Blood 76) into the appendix artery, showed a well-marked appendix lesion, together with involvement of other organs. A second animal (87) received a mixed *Staphylococcus albus* (73 and 76) into the appendix artery, and in addition to the appendix, the duodenum, the small intestine and the cecum were involved.

Of eight rabbits (55, 63, 93, 94, 65, 89, 67 and 69) which received material from cases of chronic appendicitis, four (55, 63, 93 and 67)

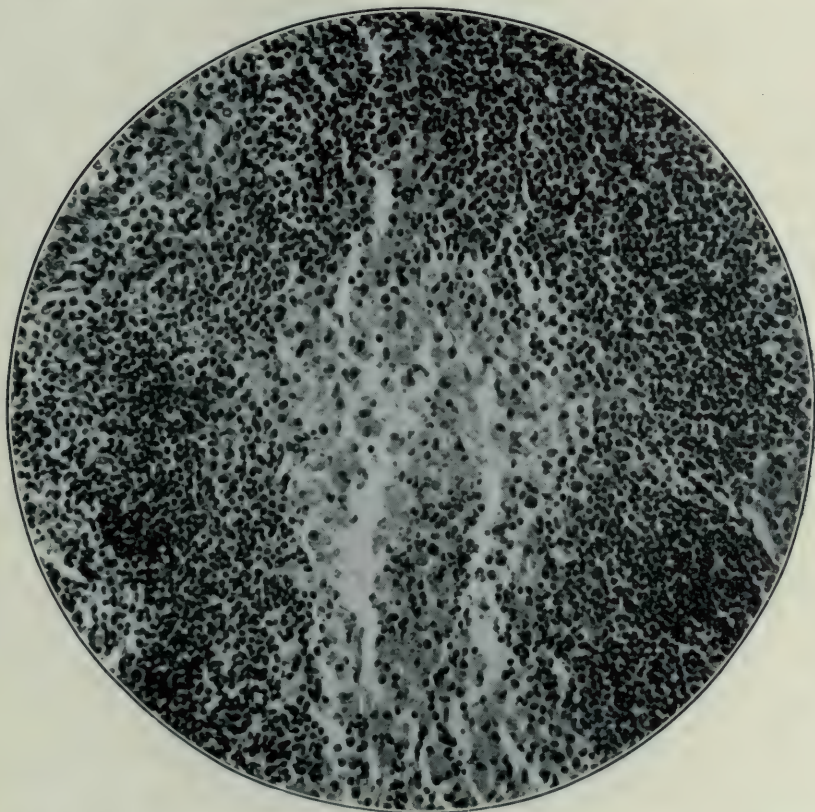


Fig. 5.—Appendix, Rabbit 59, showing necrosis of germinal center of a follicle with infiltration of leukocytes and lymphocytes.

were noted to have changes in the appendix. In the first case (B 67) one rabbit (55) was given a mixed culture of *B. acidi-lactici* and *Staphylococcus albus* and at necropsy the appendix was found affected. Subsequently, three other rabbits (63, 93 and 94) were treated with pure cultures of the *B. acidi-lactici* and two (63 and 93) developed appendiceal lesions. One rabbit (65), injected with a pure culture of the *Staphylococcus albus*, exhibited no change in its appendix. A pneumococcus recovered from the blood of rabbit 55 disclosed an unaltered appendix in rabbit 89 when this animal received a full dose

of the organism intravenously. In the second case (B 72) Rabbit 67 was inoculated with a mixture from which *B. coli-communis* and *B. xerosis* were recovered, with the result that its appendix was involved. Another rabbit (69) injected with a culture of *B. coli-communis* from a third case (B 74) of chronic appendicitis, escaped with its appendix unchanged.

The tabulated results appear in Table 2 and show the type of case, the organisms used and the frequency with which the different organs

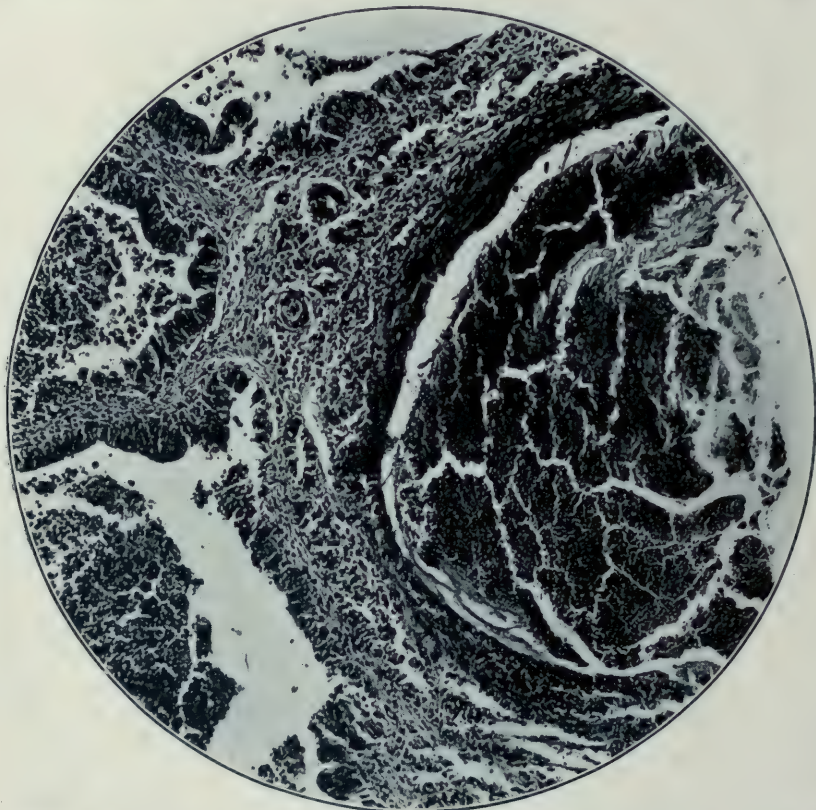


Fig. 6.—Appendix, spontaneously dead Rabbit P-13, showing a clump of necrotic debris, with red blood cells in a widely dilated gland. The lining mucosa has been destroyed and a connective tissue proliferation has taken place.

were attacked in these experiments. Of seven animals injected with the mixed cultures from the original material in Cases 9, 49, 67, 72 and 76, but three developed lesions in the appendixes. Nine animals inoculated with pure cultures of *B. acidi lactici* gave seven affected appendixes, while two mixed cultures containing *B. acidi lactici* and *B. Friedländer*, respectively, produced an appendix lesion in two rabbits. Pure cultures of *B. coli-communis* from two cases gave appendicitis in one



of four animals as against two mixed cultures containing this organism which showed an appendix in but one of two rabbits. One rabbit inoculated with a pure culture of *B. coli-communior*, and three receiving doses of a mixed culture containing this organism, developed no changes in the appendixes. Two separate cultures of *Staphylococcus albus* when given, respectively, to two rabbits resulted in the appendix being involved in but one, which was the result of a local experiment. In five animals that received doses, respectively, of three different mixed cultures containing *Staphylococcus albus*, but two had an altered appendix. These latter two animals had received material which also contained in one case a *B. acidi-lactici* and in the other a *B. Friedländer*. With the green streptococci from appendixes, two of the six animals injected with a pure culture of *Streptococcus equinus* gave an affected appendix, while all of four which received doses of two mixed cultures containing in the one *S. mitis* and in the other *S. fecalis*, were negative for the appendix. Six animals injected with cultures of a hemolytic streptococcus (*S. subacidus*) gave no appendixes. The use of the appendix material has afforded several varieties of organisms, and rabbits injected with such an array of bacteria showed the appendixes affected in our experiments in the ratio of 14 to 36, about 40 per cent. Again, where the rabbits were treated with cultures from the tonsils of appendix patients, containing mostly pure green streptococci, the appendix was involved in the ratio of 16 to 35, about 46 per cent.

#### MATERIAL FROM NONAPPENDIX CASES (TABLE 4)

A third group of fifty animals comprising those inoculated with organisms from the tonsils and adenoids of persons not affected with appendicitis, showed thirty of them with appendix lesions. Thirteen of these animals were treated with cultures made from the tonsils of fracture patients who were otherwise in good health, with the result that five exhibited altered appendixes. From seven of these tonsils a *S. viridans* was recovered. The *S. salivarius* six times associated with a *S. equinus* in one case and in the remaining tonsil a *S. mitis*. Of the seven animals injected, respectively, with the separate cultures, but two showed appendicitis, one with a *S. salivarius* and the other with the *S. mitis*. Another of the tonsils yielded a *S. anginosus*. One animal treated with the original material developed appendicitis. Subsequently pure cultures of this organism were injected into two animals, one of which only showed an altered appendix. The cultures from the remaining two tonsils gave in one case a *Staphylococcus albus* and *B. xerosis*, while in the other only a *Staphylococcus albus* was found. Two rabbits treated, respectively, with these materials had involved appendixes. The remaining thirty-seven animals of this group were treated with organisms obtained from three cases of chronic tonsillitis



TABLE 4.—RESULT OF INOCULATIONS—

Case	Source	Organisms	Rabbit No.	Rab- bits In- jected	Ap- pen- dix	Stom- ach	Heart	Lung	Joint	Skin
57	Tonsil (Colles fracture)	<i>S. salivarius</i> ; <i>S. equinus</i> ; <i>Staph. albus</i>	44	1	...	1	1	...	...	1
58	Tonsil (fracture humerus)	<i>S. salivarius</i> ; <i>Staph. albus</i>	45	1	1	1	...	1	...	1
59	Tonsil (fracture neck, femur)	<i>S. mitis</i> ; <i>Staph. albus</i>	47	1	1	...	...	1	...	1
60	Tonsil (left Pott's frac.)	<i>S. salivarius</i> ; <i>Staph. aureus</i> ; <i>B. xerosis</i>	48	1	...	...	...	1	...	1
		<i>S. salivarius</i> .....	35	1	...	1	1	1	...	1
61	Tonsil (fracture small finger)	<i>Staph. albus</i> ; <i>B. xerosis</i>	49	1	1	...	...	...	...	...
62	Tonsil (fracture ribs)	<i>Staph. albus</i> .....	50	1	1	1	1	...	...	1
63	Tonsil (fracture skull)	<i>S. salivarius</i> .....	51	1	...	1	1	1	...	1
64	Tonsil (Pott's fracture)	<i>S. salivarius</i> .....	52	1	...	...	1	1	...	1
65	Tonsil (fracture femur)	<i>S. anginosus</i> ; <i>Staph. albus</i>	53	1	...	1	1	1	...	1
		<i>S. anginosus</i> .....	57, 75	2	1	2	1	2	...	2
66	Tonsil (fracture patella)	<i>S. salivarius</i> .....	54	1	...	1	1	1	...	1
88	Chronic tonsillitis	<i>S. pyogenes-vulgaris</i> ; <i>Staph. flavus</i>	134, 135, 136, 137	4	3	3	4	3	4	3
		<i>S. pyogenes vulgaris</i>	150, 151	2	1	2	2	1	1	1
89	Chronic tonsillitis	<i>S. pyogenes-vulgaris</i> ; <i>S. salivarius</i> ; <i>Staph. aureus</i>	133, 139, 140	3	2	1	2	2	3	1
		<i>S. pyogenes-vulgaris</i>	156, 157, 162, 163	4	2	3	1	2	3	...
		<i>S. salivarius</i> .....	158, 159	2	1	2	...	1	1	...
		<i>S. salivarius</i> ; <i>S. pyogenes-vulgaris</i>	164, 165	2	1	2	...	2	1	1
		<i>S. pyogenes-vulgaris</i> (gland, Rabbit 139)	166, 167	2	2	2	1	1	1	...
89	Hypertrophy, adenoid	<i>S. pyogenes-vulgaris</i> ; pneumococcus; <i>Staph. aureus</i>	142, 143, 144, 145	4	3	2	4	4	4	2
		<i>S. pyogenes-vulgaris</i>	152, 153	2	2	2	1	1	2	1
		<i>S. pyogenes-vulgaris</i> ; pneumococcus	154, 155	2	2	2	2	...	...	...
		<i>S. pyogenes-vulgaris</i> (gland, Rabbit 144)	168, 169	2	2	2	1	...	2	1
		<i>S. pyogenes-vulgaris</i> (knee, Rabbit 145)	170, 171	2	...	2	2	1	1	...
90	Chronic tonsillitis	<i>S. infrequens</i> ; <i>B. Friedlander</i> ; <i>Staph. aureus</i>	146, 147, 148, 149	4	3	3	4	4	2	2
		<i>S. infrequens</i> (gland, Rabbit 146)	172, 173	2	1	1	1	1	...	1
	14 cases studied	.....	.....	50	30	38	33	33	25	25
91	Infected hand...	<i>S. pyogenes-vulgaris</i> ; <i>Staph. albus</i>	174, 175, 176, 177	4	2	3	2	4	...	...
	15 cases in all...	.....	.....	54	32	41	35	37	25	25

Due- de- num	Thy- mus	Mus- cle	Small Intes- tine	Brain	Cecum	Large Bowel	Pleura	Peri- car- dium	Endo- car- ditis	Peri- articu- lar	Eye	Kid- ney	Liver	Ad- renal	Cord	Gall Blad- der
...	...	...	...	...	...	...	...	...	1							
...	1	1														
1	...	...	...	...	...	...	...	...	1	...	1					
...	...	...	...	...	...	...	...	...	1							
...	...	...	...	...	...	...	1	...	...	...		1	1			
...	...	...	...	...	...	...	1	...	1							
1	...	1	...	...	...	...	...	...	1							
...	...	1	...	...	...	...	1	...	1	...	1					
1	...	1	...	...	...	...	1									
...	1	1	...	...	...	...	...	...	...	...	...	...	1			
1	1	...	1	...	1	...	1	1	...	1	1					
1	...	1	...	...	...	...	...	1								
3	2	3	2	1	2	1	2	2	...	1	1	1				
...	...	1	...	1												
2	2	1	2	2	2	2	1	2	...	1						
2	3	1	1	...	...	...	...	1	1	...	...	...	...	...	1	
2	1	...	1	...	1											
...	1	1	1	1	...	...	...	...	...	1						
1	...	1	1	2												
4	3	3	3	1	3	3	...	...	...	1						
...	1	...	...	2												
...	1	...	...	1												
1	...	1	1	2	...	...	...	...	...	...	...	...	...	2		
...	1	...	...	2	...	...	...	1								
2	3	1	4	1	2	2	...	...	1	1	...	1	...	...	...	1
1	...	1														
23	21	20	17	16	11	8	8	8	8	6	4	3	2	2	1	1
...	2	...	...	...	...	1	1	...	...	...	...	...	2			
23	23	20	17	16	11	9	9	8	8	6	4	3	4	2	1	1

and a mass of adenoid tissue from one of the patients. Here the predominant organism was a *S. pyogenes vulgaris*, and it was found that the material from these cases gave a higher number of animals with affected appendixes. Of sixteen rabbits inoculated with pure cultures of a hemolytic streptococcus, ten gave positive evidence of reaction in the appendixes. Fourteen of these animals were given doses of *S. pyogenes-vulgaris* with the result that nine developed appendicitis.

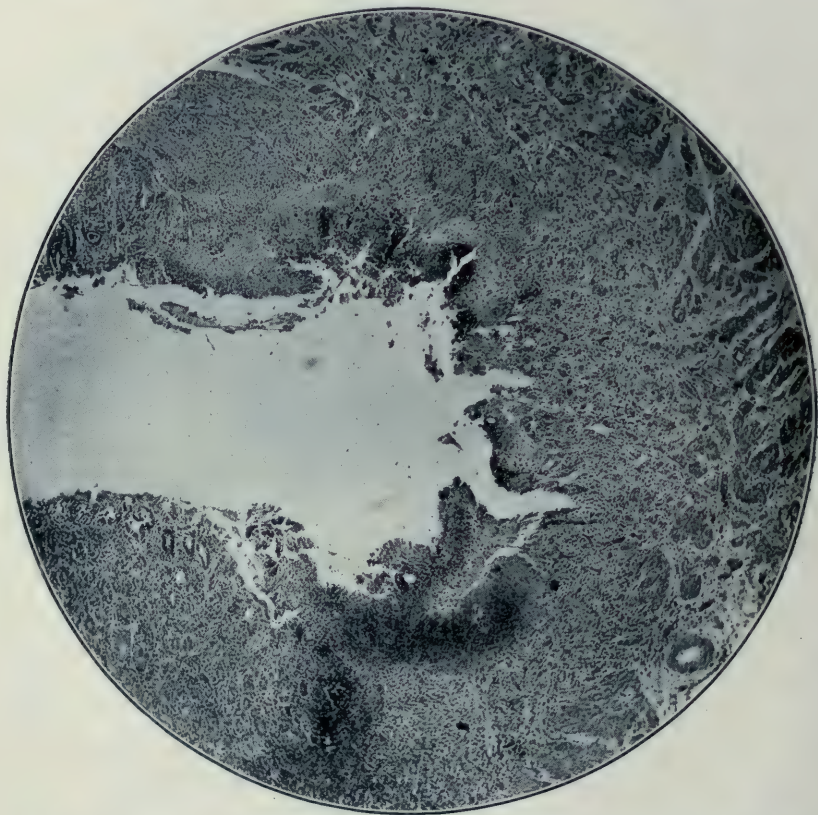


Fig. 7.—Stomach, Rabbit 69, which animal died immediately following injection, showing an ulceration in the pyloric ring, with necrosis of the mucosa and underlying tissues. Many eosinophils are scattered throughout the submucosa.

The two remaining rabbits received cultures of *S. infrequens* isolated from a gland about the base of the appendix in Rabbit 146, and one of these had its appendix involved. Four other rabbits inoculated with separate cultures of *S. pyogenes-vulgaris*, recovered from glands about the appendix of two rabbits (139 and 144), revealed changes in their appendixes. In three cases (188, 189, 190) the original material contained only one type of streptococcus, and of twelve rabbits treated



with these particular mixtures, nine had evidence of alteration in the appendixes. One of the mixtures, however, also contained a pneumococcus in addition to a *S. pyogenes-vulgaris*, and here three of four animals showed an appendicitis. This condition also occurred in the same ratio in each of two other groups of four rabbits injected, respectively, with mixtures containing in one a *S. pyogenes-vulgaris*, and in the other a *S. infrequens*. Two rabbits inoculated with a mixture of

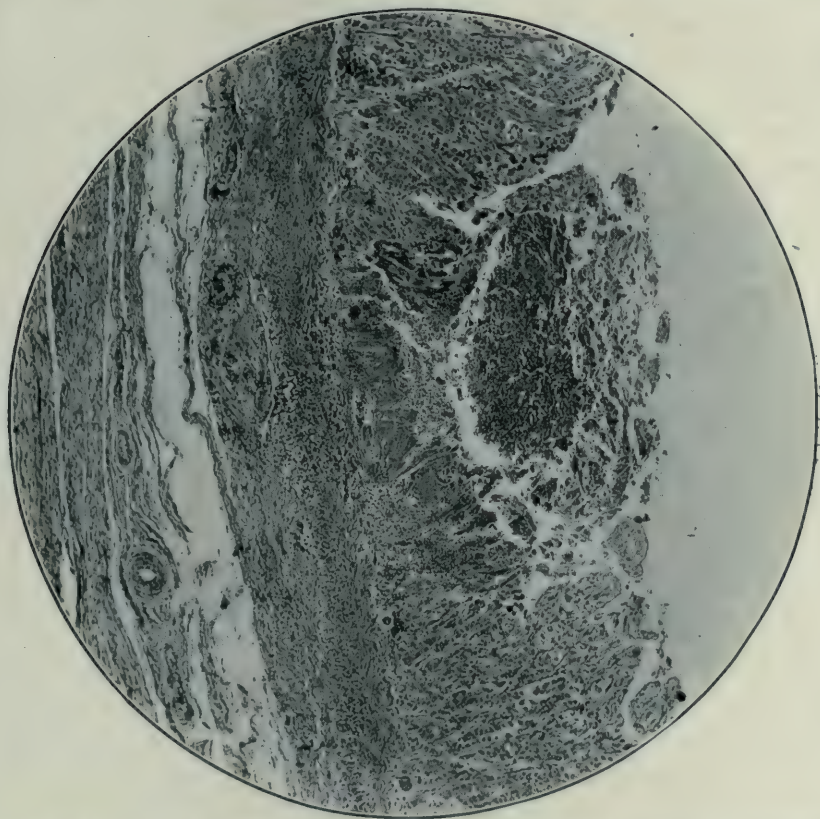


Fig. 8.—Stomach of Rabbit 135, showing ulceration and necrosis of mucosa, with hemorrhage and leukocytes scattered throughout the necrotic material. The infiltration of leukocytes extends to the outer border of the submucosa, but not beyond it.

pure cultures of the pneumococcus and *S. pyogenes-vulgaris* from Case 89 (adenoid), exhibited appendix lesions. The material from one case (89, tonsil) included both a *S. pyogenes-vulgaris* and a *S. salivarius*, and of three animals which received this mixture, two gave positive evidence of appendix affection. One of two animals injected with a pure culture of the *S. salivarius* had appendicitis, while four of six animals which received the pure *S. pyogenes-vulgaris* had

appendicitis. This is interesting in view of the fact that but one of two rabbits inoculated with a mixture of the pure isolated organisms showed an appendicitis. Of the fifty rabbits used in this group of non-appendix tonsils, forty-eight received material containing streptococci. Twenty-eight of these animals had affected appendixes. The material injected into the remaining two animals did not contain streptococci. The appendixes of both animals, however, showed hemorrhages. Thus thirty of the fifty rabbits had involved appendixes, making exactly 60 per cent.

In addition to the above group, four rabbits were inoculated with divided doses of a culture of pus from an infected hand. A *S. pyogenes-vulgaris* and *Staphylococcus albus* were recovered from the mixture. Two of the four animals showed hemorrhages in the appendix.

#### GENERAL RESULTS

A general review of the entire group of 125 animals demonstrated that the rabbits injected with hemolytic streptococci, or material including these organisms, showed a higher number of affected appendixes than those animals inoculated with green streptococci. Fifty-five animals received cultures containing hemolytic streptococci, thirty of which had an appendix lesion. Of this number there were with *S. pyogenes-vulgaris*, 23 of 36, with *S. infrequens* 4 of 6, with *S. anginosus* 1 of 3, and with *S. subacidus* 2 of 10. Forty-six rabbits were treated with material containing green streptococci, sixteen of which had appendicitis. These were divided in the following way: 13 of 34 with *S. salivarius*, 1 of 3 with *S. mitis*, 2 of 6 with *S. equinus*, while there were none of 3 with *S. fecalis*. Not only was it found that rabbits treated with hemolytic streptococci more often showed appendicitis, but that these animals also developed a more severe type of bacteremia, affecting the different organs of the body more frequently. It is therefore of the utmost importance in dealing with streptococci that the lesions produced by them should be considered in terms of the individual type of streptococcus.

Fifteen rabbits were treated with material including organisms of the capsulated gram-negative group of bacilli, and 12 were found to have appendicitis. Of this number 9, inoculated with pure cultures of *B. acidilactici*, gave 7 with appendicitis, while 1 rabbit treated with a mixture of *B. acidilactici* and *Staphylococcus albus* also had appendicitis. Four rabbits treated with a mixed culture of *S. infrequens*, *B. Friedländer* and *Staphylococcus aureus* showed 3 with appendicitis, while one rabbit treated with a mixture of *B. Friedländer*, *Staphylococcus albus* and *B. xerosis* had an affected appendix. Of the rabbits injected with material containing *B. Coli*, but 2 had appendicitis. These consisted of 1 of 4 animals inoculated with pure cultures of *B. coli*-



*communis*, 1 of 2 with mixed cultures including this organism, while all of 4 treated with material containing *B. coli-communior* were negative.

Five of six animals injected with cultures from which staphylococcus was isolated as the principal organism, revealed appendicitis at necropsy. One of these animals was injected locally into the appendiceal artery. However, the cultures at necropsy in four of the animals



Fig. 9.—Duodenum, Rabbit 117, showing extensive hemorrhage into the mucosa and about the glands in the submucosa.

showed additional organisms to those isolated from the original culture. In two cases a *B. acidi-lactici* was isolated, once from bile and once from peritoneum, while in another, *B. coli-communior* was recovered from a gland at the base of the appendix. In the fourth rabbit a *S. pyogenes-vulgaris* was found in the appendix.

A pure culture of pneumococcus produced no appendicitis in one rabbit, while mixtures of pneumococcus and *S. pyogenes-vulgaris* given to six animals showed an appendix lesion in five.



These experiments have demonstrated that the rabbit's appendix is prone to exhibit alterations in structure when organisms are administered intravenously into the blood stream. Although it was found that certain organisms were more frequently associated with such lesions than others, there was no appreciable difference in the anatomic structure of the lesions except one of degree. Of the coccaceae, the hemolytic streptococci produced appendicitis most frequently, while among the bacilli the gram-negative capsulated bacilli gave the most constant results. It may also be said of these latter organisms that they gave a larger percentage of positive results than any of the other bacteria used. The general consideration of the entire number of 125 animals shows that sixty-two, treated with a variety of organisms including both hemolytic and nonhemolytic streptococci, pneumococci, staphylococci, *B. mucosus-capsulatus* and *B. coli*, had appendicitis.

The lesions observed in the appendix consisted for the most part of bright colored hemorrhages varying in size from clustered pinpoint dots to irregular small patches 2 to 3 mm. in diameter. These areas did not tend to follow any regular distribution in the tissues of the organ, as they were at times visible from either the mucosa or serosa, while again they could not be easily seen unless the wall of the organ was sectioned. In general, however, it may be stated that the hemorrhagic foci were developed principally in the region of the deeper tissues of the wall, as in many cases small hemorrhages were observed in this region when other areas were free. Where the mucosa was chiefly affected there was found in a large number of cases a diffuse speckling of the lining, with irregularly sized hemorrhages, many of which formed small confluent patches. Over the larger areas the mucous covering was very apt to be somewhat roughened. In some appendixes the mucosa had a slightly moth-eaten appearance.

#### MICROSCOPIC APPEARANCES

Microscopically, the hemorrhages observed in the appendixes consisted of varying degrees of red-cell infiltration into the tissues of the organ beneath the mucosal lining. For the most part this reaction was present about the follicles in the submucosa, although hemorrhage quite often occurred within the follicles themselves. The hemorrhages about the follicles were found in the interstitial tissue between these structures, frequently outlining a follicle, and separating the component parts of the surrounding connective tissue. Frequently the hemorrhage was noted breaking through the connective tissue sheath of a follicle when it replaced the lymphoid cells in the immediate neighborhood. At times the red cell infiltration was quite diffuse and extended inward close to the mucosal layer. In the majority of such areas, the amount of inflammatory cell reaction was a negligible quan-

tity. That is, in the areas where the hemorrhagic infiltration was marked, inflammatory cells were usually absent. In the areas where the hemorrhage was less in amount, or absent, however, the interstitial tissue showed scattered polymorphonuclear leukocytes. In some places these cells were aggregated into fairly compact masses, with evidence of necrosis, accompanied by nuclear debris. Eosinophils were present in large numbers in the submucosal tissue of many appendixes. These

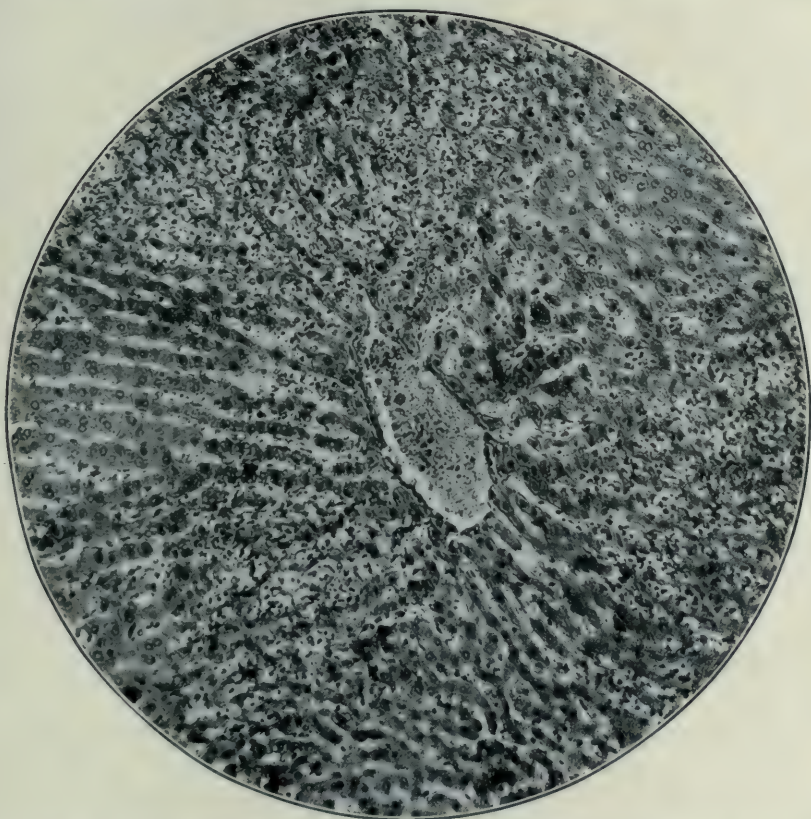


Fig. 10.—Liver, Rabbit 133, showing infiltration of leukocytes and scattered areas of necrosis about a central vein.

cells were mostly observed in the tissues about the bottom of the crypts and in the interstitial tissue between the ducts. Phagocytic endothelial cells, and at times multinucleated phagocytes, were noted in the areas of inflammatory reaction. The germinal centers of the follicles commonly exhibited proliferation of the cells. This was more readily observed where the hemorrhage was not so marked. These areas often showed slight amounts of hemorrhage with infiltration of polymorphonuclear leukocytes attended by necrosis of the proliferating cells and



scattered nuclear debris. The mucosal lining and glands were without change in all of these specimens. A number of appendixes which showed no evidence of alteration in the gross, as could be determined by hemorrhage, gave interesting microscopic pictures. The mucosal lining and gland structures were intact. In the submucosal tissue there was scattered polymorphonuclear leukocytic infiltration. At times the cells formed patchy areas with the leukocytes plugging small lymph channels. In other areas the infiltration was more diffuse, with a hyalin-like character to the involved tissue. There were clumps of debris containing leukocytes in the lumina of some glands. The lining cells of these structures were undisturbed. Again, in other sections, small areas of the mucosa were absent, with tiny capillaries and developing fibroblasts in the underlying tissue. The bases of the crypts were present with the overlying tissue loose and edematous. There was no exudate on the surface. Again, sections were observed in which the lumina of the glands were dilated by large clumps of necrotic material through which leukocytes were scattered. Most of these gland structures showed intact epithelial lining cells; however, in some places the lining cells were absent with extension of the inflammatory cells for a short distance into the submucosal tissue. Here the reaction was limited and did not have the character of acute inflammation. The prominent feature in the submucosa of these sections was the presence of many eosinophils. The follicles in the submucosa were not involved. In the most marked specimen of this type coccidia were found lying in the lumina of some of the glands, with the loss of the lining cells of the gland and the presence of many eosinophils and some leukocytes in the surrounding tissue. The same type of reaction, with the presence of coccidia, was noted in several appendixes which exhibited hemorrhages in the gross. A similar reaction was noted in the appendixes of two spontaneously dead rabbits (Fig. 6).

Six rabbits injected with sterile cultures showed interesting changes in the appendixes. These animals are not included in the series of 125 rabbits, as the appendixes exhibited no evidence of alteration in the gross. Sections of the organs showed marked proliferation of the germinal centers of the follicles. In some of these areas there was an invasion of leukocytes with necrosis of the proliferating cells. Large phagocytic endothelial cells were frequently observed in these reactions. The leukocytic infiltration occasionally broke through the capsule of the involved follicles, with the exudate coming to involve the surrounding interstitial tissue. The lymph spaces often were plugged with inflammatory cells in the region of such areas. The leukocytic infiltration extended to the submucosal tissue where there were also many eosinophils and at times small collections made up of both types of these cells. The component parts of the mucosa were free from any



sign of injury. In several places, however, leukocytes were found passing through the epithelial covering. In such places the epithelial cells were unaltered and appeared to be separated by the migrating leukocytes.

Where the injections were made into the appendiceal artery without any important interference with the blood supply, scattered areas of hemorrhage were found in the tissues of the organ. The follicles

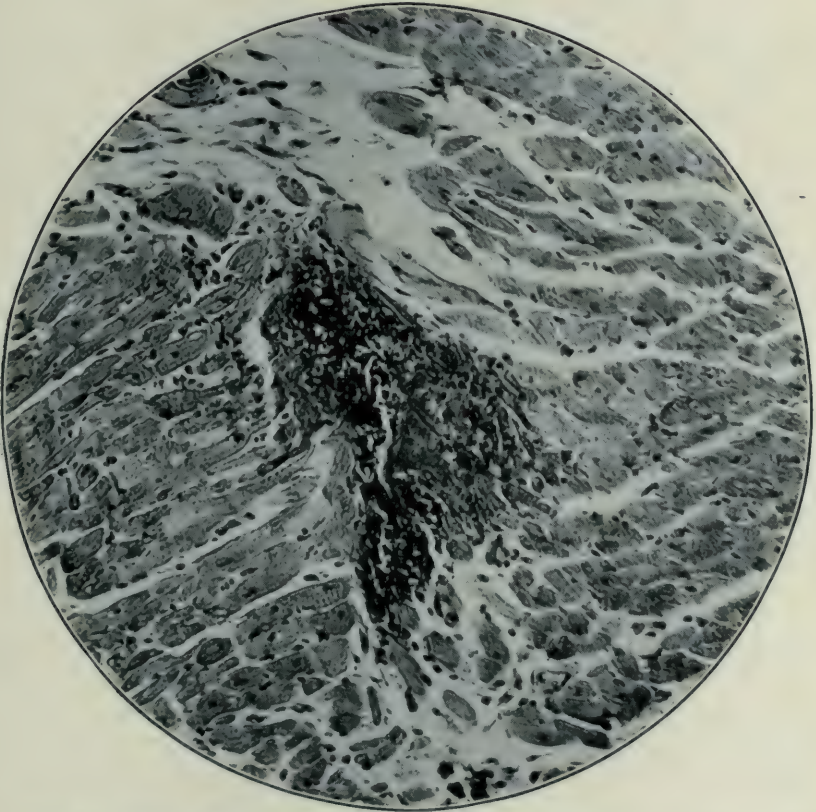


Fig. 11.—Heart, Rabbit 120, showing an area of infiltration about a clump of cocci. The infiltration consists mostly of lymphocytes, with some large mononuclears and a few leukocytes.

were almost replaced by hemorrhage, only narrow rings of surrounding lymphocytes remained. In most of the follicles hemorrhage formed the prominent feature, although in some, scattered leukocytes were observed. Large phagocytic endothelial cells were present in some of the follicles. The mucosa overlying these areas was occasionally absent. However, there was no acute exudate on the surface.

Where the blood supply of the appendix was altered at the time of

inoculation into the appendiceal artery, the organ showed marked alteration. There was an exudate on the serosa, with necrosis of the different layers of the wall and invasion of leukocytes.

The study of the rabbits' appendixes showed that where hemorrhage was the prominent feature, inflammatory cell infiltration was not marked. Proliferation of the germinal centers of the follicles with leukocytic infiltration and necrosis occurred in appendixes in which there was no evidence of hemorrhage, although the two conditions frequently occurred together. In other appendixes the submucosal tissues presented leukocytic and eosinophil cell infiltration while the follicles and mucosa were free from change. Large clumps of necrotic material were found in the ducts at times, with only occasional small areas of the lining cells absent. Coccidia were found in some appendixes with disturbance of the mucosa of the duct, and a surrounding infiltration of eosinophils and leukocytes. Sections stained by Gram's method frequently showed clumps of organisms in small capillaries, which, as a rule, were unattended by surrounding inflammation. Where the organisms were injected directly into the appendiceal artery, massive hemorrhage took place into the follicles and surrounding tissue, with some involvement of the mucosa. The presence of coccidia in some appendixes, and the fact that the reaction in the submucosa commonly did not have the typical picture of acute inflammation, would tend to show that some of the changes observed existed previous to the inoculation. In this connection the relation of *Oxyuria vermicularis* to human appendicitis may serve to explain how coccidia would act as an irritant to favor the production of appendicitis in rabbits. Only the appendixes which exhibited hemorrhages in the gross were counted. The number would be considerably more than sixty-two if those showing microscopic changes were also included. Lesions similar to those which we have observed were found in the appendixes of normal rabbits so frequently by Ghon and Namba<sup>6</sup> that they were loath to place much importance on the changes which occurred in rabbits' appendixes after the intravenous injection of streptococci, staphylococci and pneumococci. The hemorrhages which formed the prominent feature in the appendixes listed as having changes were essentially an intramural condition and were present in the majority of instances without evidence of mucosal injury. From the reactions which were noted in the other organs there is no doubt that the inflammatory infiltration in the tissues of the appendix at times depended for its origin on the organisms injected. The character of the lesions in many appendixes, however, and the presence of coccidia suggest that quite frequently a previ-

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6. Ghon and Namba: Beitr. z. path. Anat. u. z. allg. Path. (Ziegler's), 1912, 52, 130.



ously existing inflammatory reaction may have been present in the organ.

The appendix of the rabbits was not the only organ affected. Hemorrhages in the stomach, lungs, skin, and endocardium were noted more frequently than they were in the appendix. The entire gastrointestinal tract was commonly the seat of hemorrhages and presented this picture in the following order: stomach, appendix, duodenum,

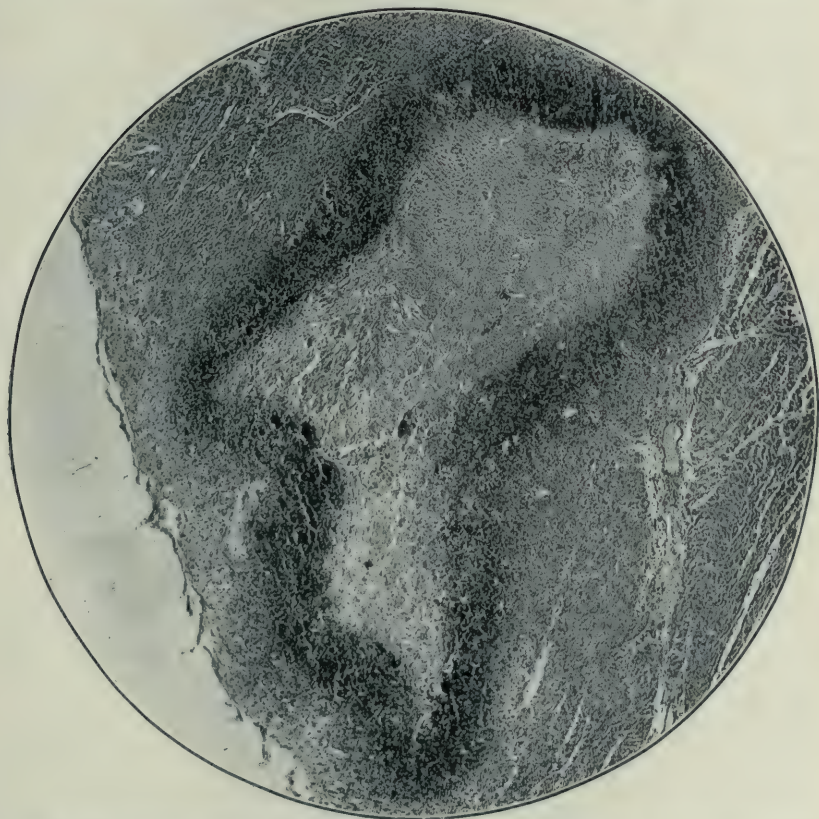


Fig. 12.—Heart, Rabbit 53, showing an infarcted area, with the inflammatory zone consisting mainly of lymphocytes.

small intestine, cecum and large bowel. Hemorrhages were also noted in the thymus, muscle, pericardium, pleurae, periarticular tissues and eye, in the order named. Almost without exception the spleen was enlarged, with tense capsule and soft deep-colored pulp. Vegetative endocarditis was found in fourteen rabbits. Where there was no hemorrhage, acute lesions were found at times in the myocardium, liver, kidney and skeletal muscles. Sometimes these areas could be recognized in the gross as small, yellowish foci. The joint fluid of the knees



was frequently cloudy, or the synovial lining quite pink, and organisms were recovered from these in forty-eight rabbits. Cultures made from the brain in forty-six cases gave twenty-one positive results. A large number of these animals showed small petechiae in the lining of the lateral ventricles, and five of the animals showed small hemorrhages in the white matter of the cord. Ten positive cultures were obtained from the gallbladder. We wish to reserve the detailed description of these changes for a separate communication. The summary (Table 5) indicates the relative frequency with which the organs were attacked in the different experiments.

The question of the experimental production of appendicitis has occupied the attention of many investigators over a long period of time. In 1885 Ribbert,<sup>7</sup> wishing to study the effect of pathogenic micro-organisms on the appendixes of rabbits, injected *Staphylococcus pyogenes-aureus* through the tip of the organ into the lumen after placing a ligature about its base. Five and one-half hours following this experiment the animal died. The appendix was found filled with

TABLE 5.—

	Rabbits Injected	Appendix	Stomach	Lung	Skin	Heart	Duodenum	Joint
Tonsils of appendix patients.....	35	16	16	16	21	12	11	10
Material from appendixes.....	36	14	28	22	23	21	19	13
Material from nonappendix cases.....	54	32	41	37	25	35	23	25
Totals.....	125	62	85	75	69	68	53	48

Explanation: Heart = hemorrhage into endocardium. Endocarditis = vegetation visible in the gross.

fluid in which large clumps of cocci were seen, and the mucosa and inner half of the follicles showed many invading organisms, while the remaining coats were free. In the same communication Ribbert claimed that he was able to demonstrate bacteria of all kinds beneath the mucous membrane of the appendix and lower ileum of normal rabbits. In this contention he was substantiated by Bizzozero<sup>8</sup> and more recently by Ghon and Namba.<sup>9</sup> Later Ribbert,<sup>7</sup> in a description of the normal and pathologic anatomy of the human appendix, called attention to the anatomic similarity between the tonsil and appendix. He said the appendix formed a blind sac, and, like the tonsil, communicated with the normally infected body cavities. Further, that just as bacteria can be forced into crypts of the tonsil during swallowing, so a like mechanical condition prevails in the appendix when the cecum is filled with feces. Stöhr<sup>9</sup> has also commented on the same points, particularly in connection with the tonsil. Roux<sup>10</sup> endeavored to produce appendicitis in an artificially made appendix in pigs by introducing foreign bodies. Finding in only one case a perforation between the appendix

7. Ribbert: Deutsch. med. Wchnschr., 1885, **13**, 197; Virchows Arch. f. path. Anat., 1893, **132**, 66.

8. Bizzozero: Centralbl. f. d. med. Wissensch., 1885, **45**, 801.

9. Stöhr: Cor.-Bl. f. schweiz. Aerzte, 1890, **20**, 537.

10. Roux: Congr. française de Chir., 1894, **8**, 213.

and large bowel, he concluded that a foreign body was not sufficient of itself to set up an inflammation.

About this time Lewis<sup>11</sup> directed attention to the apparent relationship between angina tonsillaris and appendicitis, while a little later Robinson<sup>12</sup> pointed out the fact that at times after the beginning of an acute rheumatic attack suddenly an acute typhilitis occurred, or on the other side following this acute appendicitis there was swelling of the joints of a rheumatic nature. That there appeared to be a recognized relationship between tonsillar infection, appendicitis and rheumatism at this time was evidenced by the fact that Jalaguier,<sup>13</sup> Brazil<sup>14</sup> and Sutherland<sup>15</sup> reported similar clinical observations. Jalaguier<sup>13</sup> thought appendicitis was a general infection, as he had noted acute articular rheumatism followed by perityphilitis, and had observed cases of the latter in the course of measles, mumps, typhus, varicella and scarlatina. Brazil<sup>14</sup> had observed two cases of appendicitis complicated with polyarthritis, while Sutherland<sup>15</sup> attempted to explain the coincidence of appendicitis and rheumatism by the presence of rich lymphoid tissue in the appendix, and commented further that just as the tonsil can be the starting place of an acute rheumatism, so also can the appendix be, or vice versa; an appendicitis can arise from a joint which has in turn had its infection from the tonsil. Sahli,<sup>16</sup> viewing the appendix in the light of its anatomic similarity to the tonsil, called attention to its rich supply of lymphoid

#### SUMMARY

Thy-mus	Small Intes-tine	Mus-cle	Cecum	Brain	Pleura	Peri-car-dium	Large Bowel	Endo-car-ditis	Peri-articu-lar	Eye	Kid-ney	Liver	Gall-blad-der	Cord	Ad-renal
12	6	3	3	5	2	3	1	4	5	1	4	2	2	4	
12	15	6	10	...	7	7	5	3	4	10	3	4	7		
23	17	20	11	16	9	8	9	8	6	4	3	4	1	1	2
47	38	29	24	21	18	18	15	15	15	15	10	10	10	5	2

Muscle = skeletal muscle 14, heart muscle 15.

tissue and the liability of infection of this tissue from the surface of the bowel, and he referred to a simple appendicitis as an angina of the organ. This view was supported by Nothnagel,<sup>17</sup> who said that the appendix, being rich in adenoid tissue, was as peculiarly susceptible to infection as the tonsil. Helferich<sup>18</sup> and Sunalerg<sup>19</sup> called attention to how quickly an infection invades other organs rich in lymphoid tissue, like the tonsil, neighboring tissue, lymph vessels and closely lying lymph glands. Beck<sup>20</sup> has spoken of the appendix as a large blind sac, and of the tonsil as a conglomeration of blind sacs. On account of this anatomic structure and their connection with body cavities rich in bacteria, both are subjected to the same liability of infection.

11. Lewis: Pathology of the Vermiform Appendix, London, 1893, p. 53.
12. Robinson: Med. Rec., New York., 1895, **48**, 373.
13. Jalaguier: Bull. méd., Paris., 1895, **9**, 615.
14. Brazil: Brit. Med. Jour., 1895, **1**, 1142.
15. Sutherland: Lancet, London, 1895, **2**, 457.
16. Sahli: Kongr. f. inn. Med., 1895, **13**, 201.
17. Nothnagel: Spec. Path. u. Therap., 1908, **17**, 628.
18. Helferich: Kongr. f. inn. Med., 1895, **13**, 241.
19. Sunalerg, Adrian: Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1901, **7**, 407.
20. Beck: Samml. klin. Vortr., 1898, New Series, **221**, (Chir., **65**, 1101).

Thus it is seen that even in the earliest studies of appendicitis various phases of the disease were recognized with the advancement of evidence supporting both the enterogenous and hematogenous theories. When the experimental production of appendicitis began to occupy the further attention of investigators, we find that the problem was attacked from many sides, with the result that even at present the pathogenesis of the disease is a much mooted question.

De Rouville,<sup>21</sup> who believed appendicitis had its origin from the lumen of the sac, plugged the canal of the appendix with wool, and found that the organ became diseased only as far as it was obstructed, an observation which has since been ably supported by the work of Aschoff,<sup>1</sup> Oberndorfer<sup>22</sup> and others studying human appendixes containing fecal concretions. Josué<sup>23</sup> used a streptobaccillus isolated from a case of spontaneous appendicitis in a rabbit (Charrin<sup>24</sup>) by injecting it intravenously into other rabbits, and found no localization in the appendixes of the animals. By direct injection of *B. coli* into the appendixes of rabbits, Anghel<sup>25</sup> noted an inflammation of the mucosa and a hypertrophy of the follicles in the submucosa when the organ was ligated at its base. Dominici and Letulle<sup>26</sup> produced an appendicitis in a rabbit one hour after the intravenous injection of *B. typhi*. De Klecki,<sup>27</sup> in a series of experiments, demonstrated that the virulence of the colon bacillus for the appendix can be increased under certain conditions, and also that interference with the nutrition of the appendiceal tissues can give purulent appendicitis without regard to the virulence of the organisms. Mühsam<sup>28</sup> used *B. coli*, *B. pyocyaneus* and staphylococcus locally in the appendix, and although he was able to produce certain lesions following interference with circulation by crushing and tying off the organ, he came to the conclusion that these experiments were of little value in explaining appendicitis as it occurred in humans. Beausse<sup>29</sup> concluded from his experiments that appendicitis is due to a blood infection that commonly comes from the intestinal tract, and the most important predisposing factor for appendicitis is an enterocolitis which lowers the resistance of the appendix and renders it more liable to future infection, especially by the blood.

As evidence that an acute intestinal catarrh may play a part in the production of an acute appendicitis, Ribbert,<sup>7</sup> Riedel<sup>30</sup> and Karewski<sup>31</sup> noted that such a catarrh may establish a chronic lesion in the appendix, which remains long after the intestinal condition has subsided, and may serve to favor the occurrence of an acute appendiceal attack. Oberndorfer<sup>22</sup> has also noted that this sequence of events is a very likely happening, while, on the other hand, Aschoff<sup>1</sup> was loath to take this into consideration as a forerunner of acute appendicitis.

Of the early work concerning the hematogenous origin of appendicitis, the most important was that of Adrian.<sup>4</sup> This author, recognizing the apparent clinical relationship between tonsillitis, appendicitis and rheumatism, endeavored

21. De Rouville: Compt. rend. Soc. de biol., 1896, **3**, 868.

22. Oberndorfer: Frankfurt. Ztschr. f. Pathol., 1908, **2**, 356; Ergebn. d. allg. Pathol., 1909, **13**, 527; Dis. Verhandl. d. deutsch. path. Gesellssch., 1910, **14**, 158; Verhandl. d. deutsch. path. Gesellsch., 1910, **14**, 159; Med. Klin., 1911, **7**, 2048.

23. Joséé: Compt. rend. Soc. de biol., 1897, **4**, 280.

24. Charrin: Compt. rend. Soc. de biol., 1897, **4**, 209.

25. Thése de Paris, 1897.

26. Dominici and Letulle: Semaine méd., 1897, **15**, 112.

27. De Klecki: Ann. de l'Inst. Pasteur, 1899, **13**, 480.

28. Mühsam: Deutsch. Ztschr. f. Chir., 1900, **55**, 143.

29. Beausse, Adrian: Mitt. a. d. Grenzgeb., 1901, **7**, 428.

30. Riedel: Arch. f. klin. Chir., 1902, **66**, 1.

31. Karewski: München. med. Wchnschr., 1905, **1**, 433.



to produce appendicitis in rabbits by the intravenous injection of various micro-organisms. For this purpose he employed cultures of streptococci, *Pneumococci*, *B. coli communis*, *B. typhi*, *B. tuberculosis* and *B. anthracis*. The result with all the organisms was a similar lesion consisting of inflammation, hemorrhage and necrosis of the appendiceal follicles. He was so impressed with the relative frequency of these changes in the appendix when compared with the mucosa and Peyer's patches in the remainder of the bowel, that he concluded the appendix of rabbits and humans, owing to the rich adenoid tissue, forms a favorable place for the lodgment of bacteria, just as the joint, the serous lined cavities, lymph glands or other organs supplied with a rich lymphatic apparatus. In this con-

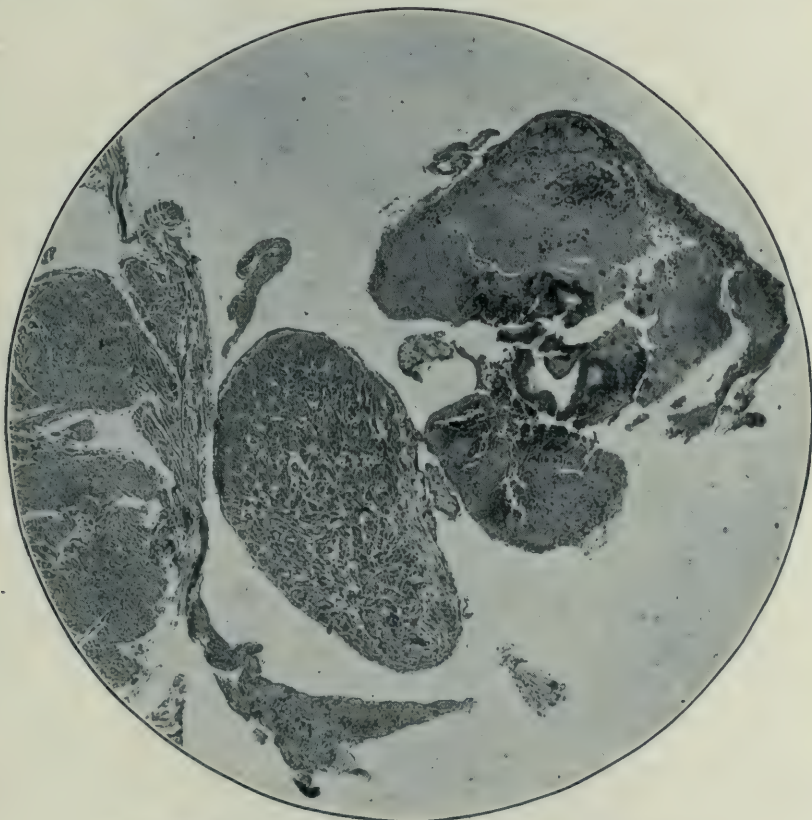


Fig. 13.—Rabbit 48, showing a vegetation on mitral valve.

nection we find that Mori<sup>32</sup> injected streptococcus, *Staphylococcus aureus*, *B. proteus* and *B. coli* into the superior mesenteric artery, and observed other parts of the intestine showing lesions similar to those in the appendix. Mori also states that he found spontaneous appendicitis in laboratory animals.

Again, in 1906, Kretz<sup>2</sup> attempted to prove that the small hemorrhages observed in the mucosa of human appendixes are the initial stage of the virulent type of acute appendicitis as it is observed at operation. Kretz maintained that the infection came by way of the blood from the tonsils, which did not need to

32. Mori: Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1903, **12**, 639; Deutsch. Ztschr. f. Chir., 1904, **73**, 123.

exhibit a recognizable inflammation clinically. He based this contention entirely on the fact that he found fibrin in the blood and lymph vessels of the tonsil along with lymphocytes and bacteria. In this connection, Meyer,<sup>33</sup> a student of Aschoff, observed the same condition in twelve of thirty-one pairs of tonsils from patients dying of all causes. From this evidence Aschoff was inclined to believe that these alterations were due to postmortem change. Kretz<sup>2</sup> said that streptococci from the tonsil located in the follicles of the appendix near the germinal center, and that he found streptococci there in pure cultures. When these organisms lodge within the capillaries of the germinal centers of the follicles, they multiply and destroy the capillary wall with resultant hemorrhage and destruction of the follicle, the process finally rupturing through the mucous membrane. The process either ends here or goes on to complete disintegration of the follicle with fibrinous exudate and formation of a crater-like ulcer, from which last condition extensive inflammation of the appendix may occur.

Following the statement of Kretz,<sup>2</sup> pointing to a specific infective agent in appendicitis, a storm of dissent arose among the German authors. Aschoff<sup>1</sup> was chief among those who combated the theory of Kretz, and held that the disease started as an enterogenous infection at the bottom of the crypts, where even in the earliest hours (5) following the outset of the symptoms, the epithelium shows a small accumulation of fibrin and leukocytes. Beneath the fibrin clump, and in the neighboring tissue, many polynuclear leukocytes are found with the neighboring lymph vessels taking part in the inflammation. The leukocytic infiltration advances and finally invades the muscularis and subserosa, the extension of the inflammation into the deeper tissues being in the form of a wedge with the apex at the inflamed crypt. In the fibrin overlying the surface, there are in the earliest stages many bacteria, most of which are intracellular in the form of gram-positive bacilli and diplococci. The primary defect is multiple in most cases. Confluence of the individual areas is very common, leading to phlegmonous infiltration of the appendix. Following this, there may be a breaking down of the muscularis, with the development of abscesses in the wall, going on in some cases to perforation and in others to extensive mucosal ulceration, with a possible destruction of the entire organ.

Aschoff was of the opinion that the areas of hemorrhage which occur in appendicitis are traumatic, as they occur in appendixes where there is no evidence of inflammation whatsoever. In this view he was supported by Fränkel.<sup>34</sup> Although Oberndorfer<sup>22</sup> considered most of the hemorrhages observed by Kretz<sup>2</sup> as being of traumatic origin, he nevertheless said that septic embolic disease of lymph follicles is possible, as he found this lesion in the follicles of the intestine, attended by hemorrhage. Albrecht<sup>35</sup> has discussed similar findings to those of Oberndorfer; he, however, considers the hemorrhages as of traumatic origin, as he has observed fresh hemorrhages in all types and stages of appendicitis. Riedel<sup>36</sup> looked on the areas of hemorrhages as a part of a chronic catarrh which brings about injury to the mucosa, permitting the entrance of infecting agents into the finer lymph vessels, with the subsequent development of an acute inflammation in the appendix, similar to the lymphangitis of the skin seen in erysipelas. The severity of the reaction depends on the invading organisms.

That a rapidly developing lymphangitis can develop in the appendix was supported by Heile,<sup>36</sup> who noted that the rich lymphoid structure of the appendix favors an invasion from a primary epithelial defect, as in the very first hours of an attack there is an exudate on the peritoneum consisting of leukocytes,

33. Meyer and Aschoff: *Verhandl. d. deutsch. path. Gesellsch.*, 1907, **11**, 313.

34. Fränkel: *Dis. Verhandl. d. deutsch. path. Gesellsch.*, 1904, **8**, 246. Oberndorfer: *Med. Klin.*, 1911, **7**, 2048.

35. Albrecht: *Dis. Verhandl. d. deutsch. path. Gesellsch.*, 1907, **11**, 318; *ibid.*, 1910, **14**, 158. *Wien. klin. Wchnschr.*, 1909, **22**, 1359.

36. Heile: *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1911, **22**, 58; *ibid.*, 1913, **26**, 345.



fibrin and bacteria. Heile further goes on to say that as a streptococcus can be caught in the deep tissues of the follicular tissue of the tonsil and set up an inflammation, the same occurrence is also noted in the appendix. Nowicki<sup>37</sup> believes that infection of the tonsil and appendix are similar and occur in a like manner, and points out that Teichmann<sup>38</sup> has demonstrated the presence of large lymph vessels in the appendix wall which send branches into the submucosa, forming a network about the follicles. Golubof<sup>39</sup> described appendicitis as a

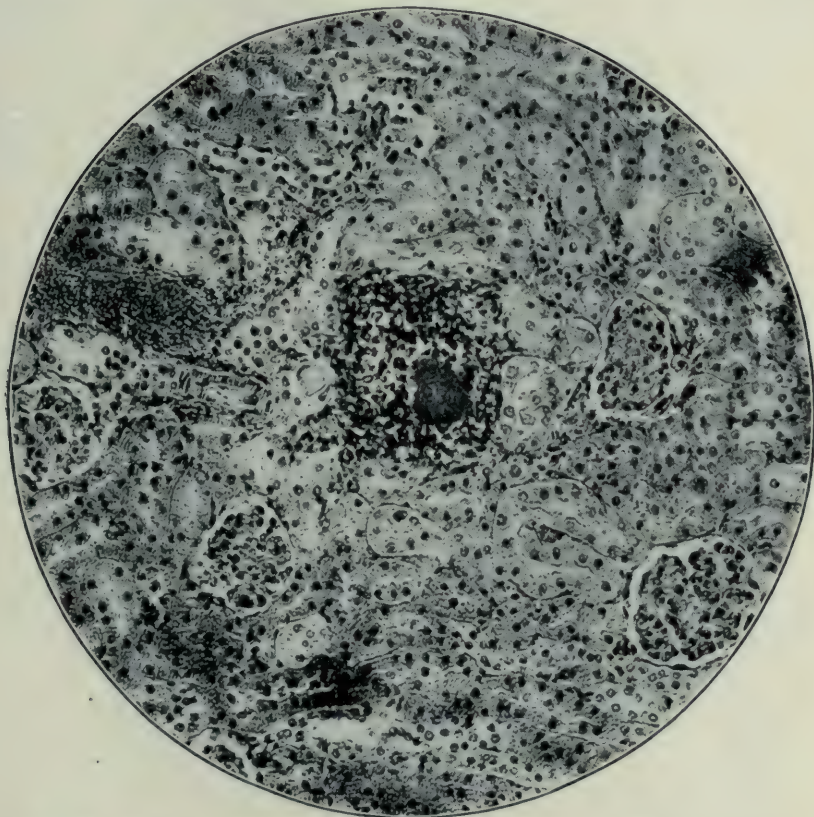


Fig. 14.—Kidney, Rabbit 64, showing embolic plugging of the glomerulus and infiltration of lymphocytes and leukocytes.

disease of the organ, arising *sui generis*, and cites in comparison follicular tonsillitis and dysentery of the large bowel. The enterogenous theory for the production of appendicitis has been further supported by Oberndorfer,<sup>42</sup> Albrecht,<sup>43</sup> Oguro,<sup>40</sup> Weichselbaum,<sup>41</sup> Beitzke,<sup>42</sup> Lubarsch,<sup>43</sup> Schmorl<sup>44</sup> and many others.

37. Nowicki: Virchows Arch. f. path. Anat., 1909, **195**, 175.

38. Teichmann and Nowicki: Virchows Arch. f. path. Anat., 1909, **195**, 175.

39. Golubof: Berl. klin. Wehnschr., 1897, **1**, 9.

40. Oguro: Virchows Arch. f. path. Anat., 1909, **197**, 548.

41. Weichselbaum: Dis. Verhandl. d. deutsch. path. Gesellsch., 1910, **14**, 156.

42. Beitzke: Dis. Verhandl. d. deutsch. path. Gesellsch., 1910, **14**, 155.

43. Lubarsch: Dis. Verhandl. d. deutsch. path. Gesellsch., 1910, **14**, 157.

44. Schmorl: Dis. Verhandl. d. deutsch. path. Gesellsch., 1901, **14**, 157.



Aschoff<sup>1</sup> recognized that in many cases tonsillitis and appendicitis occur simultaneously, one after the other, or even result one from the other. He nevertheless stated that the appendix may well be infected enterogenously from the tonsil. An important objection to the hematogenous theory, and the one which Kretz<sup>2</sup> admitted was the most serious, was that in none of these cases was he able to demonstrate the offending agent in the blood. This finding has been substantiated by the studies of Libmann,<sup>45</sup> Simmonds,<sup>46</sup> Lubarsch,<sup>43</sup> Fränkel<sup>44</sup> and others on the blood of patients suffering from acute appendicitis. Kretz<sup>2</sup> maintained that the blood infection was only transient in nature, and at the time of the general infection there was no doubt that many other organs are affected, and calls attention to the transitory bacteremias which lead to an endocarditis, or to an osteomyelitis, where the condition remains mostly a solitary infection. In reply to this view of Kretz, Lubarsch<sup>43</sup> found with regard to the relationship between angina and appendicitis that clinical statistics do not prove a relationship between the two, in view of the extraordinary frequency of angina over appendicitis. He further goes on to say that one can hardly prove that the organisms associated with an angina are identical with those causing the appendicitis, unless the offending agent can be demonstrated in the blood, which event should be possible in at least some cases. Albrecht<sup>45</sup> made the very significant comment that since there are enormous numbers of streptococci which normally inhabit the entire gastro-intestinal canal, it does not follow that streptococci which can be demonstrated in the appendix necessarily come by way of the blood stream. Oberndorfer<sup>22</sup> pointed out the similarity between the tonsil and appendix, in that the epithelial covering of the follicles and crypts is not as efficient as in other parts of the gastro-intestinal tract, permitting a continual wandering of lymphocytes through the epithelium, which must be considered of great importance in the entrance of bacteria.

This same author remarked in regard to the Kretz theory of appendicitis that without doubt in some of the early cases a part of the follicles may be missing. There is also a pseudomembrane with necrosis at the bottom of the crypts, together with typical surface alterations. In a study of septic embolic hemorrhagic enteritis where the blood was overwhelmed with bacteria and septic emboli, he found that metastases occurred in the intestine, with a predilection for the follicular apparatus, while in the same cases the appendiceal follicles were frequently free of any inflammation. He further points out that if appendicitis is a hematogenous infection of the follicles, the remaining follicles of the small and large intestine, on account of their anatomic similarity to those in the appendix, should show similar alterations more frequently, and the fact that the follicular apparatus of the intestine of individuals dying of appendicitis is free, forms the strongest argument against the theory of Kretz.

A septic embolic infection of the appendix was not denied by Aschoff, and he spoke of its occurrence in certain cases. However, Oberndorfer<sup>22</sup> and Schmorl<sup>44</sup> have commented on the relative infrequency with which the appendix is attacked in cases of pyemia, when the other organs and upper gastro-intestinal tract developed metastatic changes. Routier<sup>47</sup> pointed out that the appendicitis which occurs in connection with angina, measles, varicella, parotitis, enteritis or enterocolitis, does not preclude the fact that most cases are a primary condition in the appendix. Wette<sup>48</sup> observed hematogenous infection of the appendix in pneumonia, angina and carbuncle, while Schrumpf<sup>49</sup> has described embolic plugging of the appendiceal vessels by an embolus in heart disease.

45. Libmann and Aschoff: *Verhandl. d. deutsch. path. Gesellsch.*, 1907, **11**, 313.

46. Simmonds: *Dis. Verhandl. d. deutsch. path. Gesellsch.*, 1906, **10**, 234.

47. Routier, Adrian: *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1901, **7**, 407.

48. Wette: *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1906, **16**, 303.

49. Schrumpf: *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1907, **17**, 167.

Jores<sup>50</sup> has reported two cases of metastatic appendicitis developed during the course of fatal scarlet fever, where there was an infiltration in the outer coats of the organ without the mucosal changes characteristic of ordinary appendicitis. In a rabbit injected subcutaneously with urine, Gouget<sup>51</sup> found the development of a pyemia, with localization in the lymphoid structures, producing an abscess in spleen, suppurating mesenteric lymph nodes, and an appendix which showed nodules in the wall representing follicles crammed with leukocytes. Aschoff<sup>1</sup> remains of the opinion that human appendicitis is mostly a primary mucosal

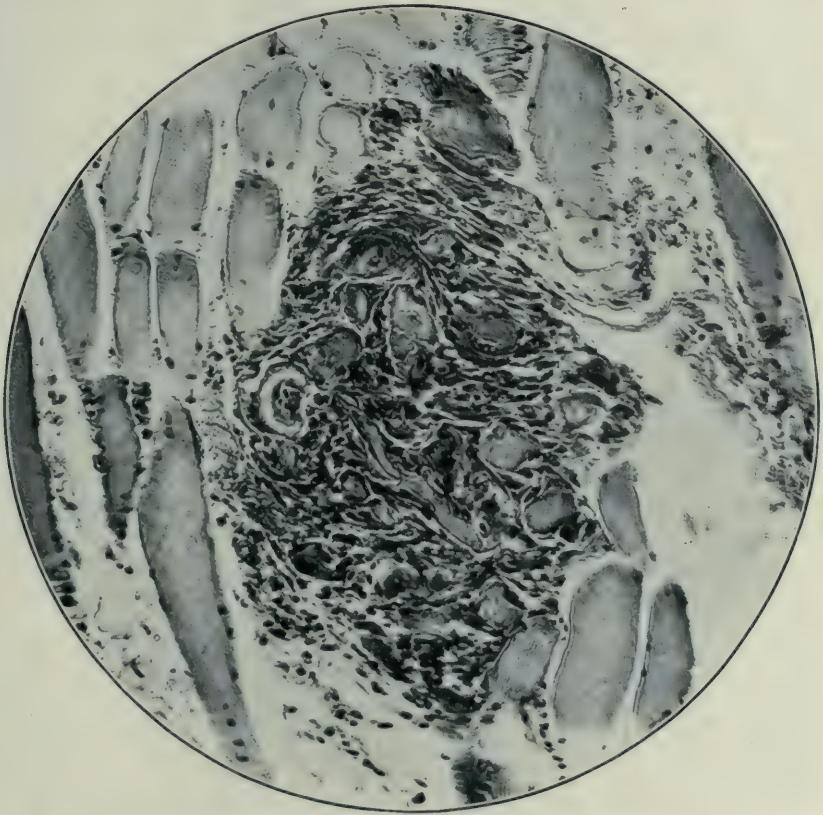


Fig. 15.—Skeletal muscle, Rabbit 157, showing an area where there is proliferation of large, elongated cells between the muscle bundles, causing necrosis of the ones immediately involved. Lymphocytes and an occasional leukocyte are observed.

infection. The type of appendicitis described by Kretz<sup>2</sup> forms a particular variety which is easily distinguished clinically, in that the patients exhibit evidence of marked sepsis, and speedily die from other lesions following operation for the appendix condition.

More recently, in regard to the experimental production of appendicitis,

50. Jores: *Verhandl. d. deutsch. path. Gesellsch.*, 1913, **16**, 197.

51. Gouget: *Compt. rend. Soc. de biol.*, 1899, **1**, 631.



Poynton and Paine<sup>52</sup> have produced appendicitis in rabbits by injecting intravenously an organism isolated from the appendix of a patient suffering with angina and acute polyarthritis. The organism recovered from the throat had similar cultural and biologic characteristics to the one recovered from the appendix; however, this organism produced only arthritis in rabbits. Tedesco<sup>53</sup> found leukocytic infiltration, necroses and hemorrhage in the follicles of rabbits' appendixes when the animals were injected intravenously with staphylococcus, streptococcus and *B. anthracis*. Heile,<sup>54</sup> in an effort to demonstrate a specific action of bacteria on the appendix when intravenously injected, found that they did not tend to localize in the appendix more than in other organs. In this respect he was unable to corroborate the work of Adrian.<sup>4</sup> Again, Ghon and Namba<sup>5</sup> observed that rabbits injected intravenously with streptococci were very prone to show these organisms in the vessels of the appendiceal follicles, without evidence of inflammation. This fact was noted in ten of fourteen animals. However, they were not inclined to consider the lesions present as very important, in that these were in all respects similar to those which they had observed in the normal rabbit appendixes. Of fifty-five normal rabbits examined by them, they reported that thirty-seven showed gross appendiceal lesions, while in the remaining eighteen there were some which showed microscopical changes. Charrin,<sup>54</sup> Josué<sup>55</sup> and Mosny<sup>54</sup> have also noted spontaneous appendicitis in the laboratory animals.

From the foregoing review of the literature it is evident that for a long time there has been recognized an apparent relationship between angina and appendicitis, with the result that several investigators (Adrian<sup>4</sup> Kretz,<sup>2</sup> Poynton and Paine<sup>52</sup>) have endeavored to prove that appendicitis occurs by a hematogenous infection from the tonsil. Apolant<sup>55</sup> saw three cases of appendicitis in conjunction with angina and thought that the organisms entered the tonsil and from there sought the *locus minoris resistentiae*. The controversy between those supporting the hematogenous theory and those of the opposition in favor of the enterogenous origin of appendicitis has waged fervently without a final decision.

Recently, Rosenow<sup>3</sup> has published extensive results concerning the experimental production of appendicitis in rabbits, and his work has added a new conflicting attitude to the subject. Apparently following the idea of Kretz,<sup>2</sup> that a streptococcus from the tonsil localizes in the appendix follicles, Rosenow has still further endeavored to prove that such an organism has an elective affinity for the appendix and seeks the tissues of this organ by special predilection. Not only has this property been attributed to streptococci, but colon bacilli and staphylococci possess similar characters under the proper conditions. It has been specified that these organisms must be obtained from the appendix itself, or from the tonsils of such patients, and be cultured in a special medium containing ascites fluid. Further, the medium must be used in tall columns in order to afford a gradation of oxygen tension, which is essential for the organisms to retain the elective qualities. Several authors have reported the selective localization of organisms in tissues. Bezancon and Labbe<sup>56</sup> found that a staphylococcus from a cutaneous abscess which had localized in a traumatized joint, showed a

52. Poynton and Paine: Lancet, London, 1911, **2**, 1189; *ibid.*, 1912, **2**, 439; Proc. Roy. Soc. Med., 1911, **5**, 18; Researches on Rheumatism, New York, 1914.

53. Tedesco: Arb. a. d. Geb. d. path. Anat. u. Bakter., 1908, **6**.

54. Mosny: Compt. rend Soc. de biol., 1897, **4**, 241.

55. Apolant: Therap. Monatsch., 1897, **1**, 9.

56. Bezancon and Labbe: Compt. rend. Soc. de biol., 1900, **2**, 31.



tendency to localize in the joints of other animals on intravenous injection for several generations after isolation from the traumatized joint. Forssner<sup>57</sup> obtained a streptococcus from an axillary abscess, and cultured it in extracts of kidney, and in kidney tissue, both in vitro and in vivo. The original organism exhibited no tendency to localize in the kidneys on intravenous injection, while the one procured after passage through kidneys presented a marked tendency to produce lesions of the kidneys on intravenous injection. After the last animal passage, the streptococcus retained this property on serum broth for four or five generations, when it was lost. Strains of *B. pestis* repeatedly grown in lung tissue show an increased ability to cause pneumonic lesions on intravenous or subcutaneous injections. In contradistinction to these facts, Irons, Brown and Nadler<sup>58</sup> were unsuccessful in attempts to obtain a return of invasive power for tissues of the eye in strains of streptococcus which had lost it, by growing the organisms in the living eye.

From the foregoing evidence it is difficult to conceive in what manner the tonsils act to impress such varied affinities on organisms, and how a transudate from the peritoneum may serve to preserve these qualities.

We wish to call attention to the fact that the medium used for this work was dextrose serum broth, used in 150 c.c. amounts, in Erlenmeyer flasks. In 125 rabbits injected intravenously with a variety of organisms cultured by this method, gross hemorrhages were noted in the appendix sixty-two times. The fact that the organisms injected can frequently be demonstrated in the hemorrhages which occur in this organ, is not conclusive evidence that these organisms select the tissues of the appendix with a predilection. When a gross hemorrhage takes place into the tissues of an organ, whatever material is in the blood will be carried into the involved area. If the blood is overwhelmed with bacteria, many of the small vessels in various parts of the body become plugged. This plugging of small vessels forms a mechanical obstruction to the circulation, and the delicate capillaries are very apt to rupture, followed by hemorrhage, which must be composed of all the constituents of the blood. The frequency with which hemorrhage and bacterial emboli are observed without the presence of an inflammatory reaction, suggests that a mechanical factor must be considered as well as the direct action of the organisms and their toxins.

We have also observed that the thymus, lymph glands of the mesentery, of the inguinal region, glands about the head of the cecum, and also Peyer's patches, were commonly affected in conjunction with hemorrhages distributed variously throughout the organs of the body. From these findings and the evidence which we are able to gather from the literature it would appear that the rabbit is peculiarly prone to exhibit alterations in his lymphoid apparatus when organisms gain entrance to his blood stream. It is a generally recognized fact that

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57. Forssner, Irons, Brown and Nadler: Jour. Infect. Dis., 1916, **18**, 315.

58. Irons, Brown and Nadler: Jour. Infect. Dis., 1916, **18**, 315.

there is no tissue or organ of the human body immune to invasion by streptococci. This organism is not alone confined to the human species, but is also found associated with diseased conditions in domestic animals. Streptococci have been indiscriminately found associated with erysipelas, puerperal fever, septic sore throat, scarlet fever, malignant endocarditis, polyarthrititis, streptococcic pneumonia, purulent pleurisy, summer diarrhea of children, together with wound infection and sepsis, with a host of other conditions to which human beings are susceptible. In animals they have been found with spontaneous infections, puerperal fever, contagious coryza, pleuropneumonia of horses and certain inflammatory diseases of the udders of cows. Erysipelas has been induced experimentally with matter taken from wounds, from throats in angina and from the uterus in puerperal fever. Further, as Vaughan<sup>59</sup> has said, all these and many other diseases are due to the same organism, and are mutually convertible one into another. In this connection, Poynton and Paine<sup>62</sup> have produced arthritis in rabbits with streptococci from the throat, feces and urine.

If appendicitis is a local manifestation of a general infection due to a special organism, it should be possible to demonstrate the organism in the blood at some time during the course of the disease. Until this fact has been definitely established for appendicitis, not including the pyemic type of the disease, one can little hope conclusively to prove that a particular organism is at fault. Kotzenberg,<sup>60</sup> in a study of the opsonic index of appendix patients, found that their serums reacted equally well for streptococci, staphylococci and *B. coli*. Further, the fact that a variety of different organisms have been associated with changes in rabbits appendixes, tends to indicate that a special organism has not yet been found. The production of appendicitis in animals by the intravenous injection of large doses of vigorous bacteria cannot be considered as an indication that these organisms attack the human appendix by way of the blood. Even in the most extreme cases, where the blood is overwhelmed with bacteria, the appendix is only occasionally involved (Oberndorfer,<sup>22</sup> Albrecht<sup>35</sup>). The condition produced in the appendix of the rabbit is primarily intramural, and is the analogy of a particular type of human appendicitis observed in pyemia. It cannot be compared with the common type of appendicitis as described by Aschoff.<sup>1</sup> At present all authors are agreed that infection is requisite in the production of appendicitis. However, the widely divergent theories which have been advanced, including stasis (Dieulafoy), the hematogenous (Kretz<sup>2</sup>) and alimentary theories (Aschoff<sup>1</sup>), the idea of the similarity between the tonsil and appendix (Sahli<sup>16</sup>), and lastly,

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59. Vaughan: Commemoration Volume, 1915, p. 107.

60. Kotzenberg and Much: Deutsch. med. Wchnschr., 1909, 5, 201.

the effect of fecal concretions (Sprengel<sup>61</sup>), indicate that the appendix is the subject of many insults. Further, the comparison of the lesions which occur in the appendixes of animals in this type of experiment with human appendicitis, should be made with extreme caution, as the disease in the animal is produced by very vigorous methods, and never assumes the character of the disease as it is observed in man. We feel obliged to protest against the idea that any organism possesses a peculiar affinity for any given organ when the same organism shows a capacity for the invasion of other organs in a very appreciable proportion of experiments. As the result of our study of the literature, and our own experimental work, we find little evidence to support the belief that appendicitis in the human subject is ordinarily caused by a blood infection.

This work was carried out under the direction of Dr. John A. Hartwell, to whom I am much indebted for advice and assistance.

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61. Sprengel and Oberndorfer: *Ergebn. d. allg. Pathol.*, 1909, **13**, 527.



## TRANSIENT HEART BLOCK—ELECTROCARDIOGRAPHIC STUDIES \*

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The study of the heart beat by means of modern instruments of precision, such as the polygraph and the string galvanometer, has revealed the fact that the graver forms of cardiac arrhythmia, as heart block and auricular fibrillation, are much more common than was previously suspected. It has also been recognized that such arrhythmias as paroxysmal tachycardia, flutter and premature contractions (extrasystoles), are notoriously transient in most cases, but until very recently heart block (especially complete block) and auricular fibrillation were looked on as being nearly always permanent conditions due to extensive organic changes. In 1910, however, I<sup>1</sup> was able to show that complete heart block may exist for years without demonstrable lesion of the bundle of His at autopsy, and similar cases have occasionally been reported since that time (see Ref. 8). In the case of auricular fibrillation, I have recently shown<sup>2</sup> that transient attacks are not only fairly common, but may be divided into three well-defined groups, whereas transient heart block occurring during an acute infection (Naish and Kennedy<sup>3</sup>), or as the result of digitalis medication (Hewlett<sup>4</sup>), is now an even better recognized condition. It is to such cases that Hart<sup>5</sup> has applied the term "functional heart block," "not because we believe organic changes are absent, but because such changes are of such a moderate degree or are of such a nature that by the administration of drugs the evidence of functional abnormalities can be considerably modified." With these reservations the term is a serviceable one, and yet it would be unfortunate to distract attention too much from the myocardial damage, whether acute or chronic, slight though it may be, which undoubtedly underlies the majority of such cases. The following cases illustrate different types of this rather complex condition.

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\*From the Medical Division and the Pepper Clinical Laboratory, Hospital of the University of Pennsylvania.

1. Krumbhaar, E. B.: Adams-Stokes Syndrome, with Complete Heart Block without Destruction of the Bundle of His, *THE ARCHIVES INT. MED.*, 1910, **5**, 583.

2. Krumbhaar, E. B.: Transient Auricular Fibrillation, *THE ARCHIVES INT. MED.*, 1916, **18**, 263.

3. Naish, A. E., and Kennedy, A. M.: Heart Block in Acute Rheumatic Carditis, *Lancet*, London, 1914, **2**, 1343.

4. Hewlett, A. E.: Digitalis Heart Block, *Jour. Am. Med. Assn.*, 1907, **48**, 47.

5. Hart, T. S.: Functional Heart Block, *Am. Jour. Med. Sc.*, 1915, **149**, 62.

*I. Transient Partial A-V Block in Acute Rheumatic Carditis.*—The case selected as an example of this type of block was observed during a recurrence of acute articular rheumatism. It illustrates the temporary nature of the block, a parallelism between the block and the degree of rheumatism, the type of ventricular arrhythmia produced, and the influence of vagus stimulation and inhibition on the disturbed mechanism.

CASE 1.—M. C., single, white, actress, aged 34, was admitted to the medical service of the University Hospital, Feb. 19, 1915, complaining of "an acute attack of rheumatism" of two weeks' duration, following tonsillitis and exposure. Severe pains occurring simultaneously in several joints and in most of the muscles of the body, were partially relieved by salicylates, but still persisted in the back, sides and arms. The wrist and elbow of the left arm were painful, tender, red and swollen.

When 16 years old the patient had had a severe attack of rheumatic fever, with cardiac complications, and since then she had had several recurrent attacks, and had experienced more or less palpitation and dyspnea. Her profession exposed her to inclement weather and she was addicted to excess in

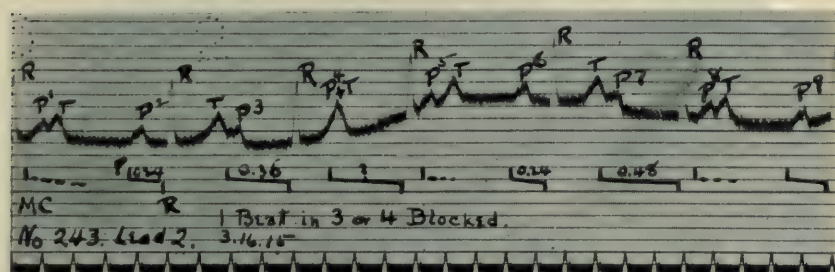


Fig. 1.—Case 1. Electrocardiogram (Lead 2) of M. C., showing partial heart block. It will be noticed that the first, fifth and eighth P waves fail to be followed by a ventricular complex. After each such "dropped" beat, the succeeding P-R interval is almost within normal limits, but the P-R interval of each succeeding cycle is lengthened until another "dropped" beat occurs. In this, as in other electrocardiograms of this series, platinum wire strings were used with resistances varying between 3,000 and 4,000 ohms. The tension of the string was so standardized that a change in potential of 1 millivolt caused a deflection of 1 cm. As the string could not be standardized with the patient in circuit, 1,400 ohms were added as an arbitrary equivalent of the patient's resistance. Time intervals are expressed by a Jaquet time marker in fifths of seconds, and occasionally by vertical lines indicating  $\frac{1}{5}$  and  $\frac{1}{25}$  second.

eating and late suppers, with immoderate indulgence in beer, whisky and coffee. Several other members of the family suffered with "rheumatism." At no time during the present illness had the patient received any digitalis or allied drugs.

*Physical Examination and Course of Illness.*—The condition of the muscles and joints corresponded to the description given by the patient. Dental caries, pyorrhea alveolaris, general nervousness and the state of the heart were the other points requiring consideration. The heart dullness began above at the third rib, the right border was 3 cm. to the right of the midsternal line, and the left border 12 cm. to the left of the midsternal line and 2 cm. outside the midclavicular line. There was a well-marked apex beat in the fifth space, 2 cm. inside the limit of dullness. At the apex was heard a loud, harsh, blowing systolic mur-







of the P-R interval, until, after about every fourth auricular impulse, the ventricle fails to respond. Following such a "dropped" beat, the P-R interval is shortened and a similar cycle recommenced. In Leads 1 and 3 the degree of block is different, there being a 2:1 rhythm in which every other auricular contraction fails to be followed by a ventricular contraction. These findings confirmed polygraphic tracings taken on the same day. Contrary to expectation, during vagus stimulation by ocular pressure the degree of block was slightly less. The "dropped beats" occurred as before, but with lesser frequency, and the preceding P-R intervals, though prolonged for a short time to more than 0.4 second, were all equal for several beats. Electrocardiograms taken on the next two days revealed the same state of affairs. At this time, 1 mg. of atropin administered hypodermically proved sufficient temporarily to prevent the "dropping" of beats. The P-R interval, however, remained prolonged (0.38 second) and there was no appreciable rise in the auricular rate. After four days

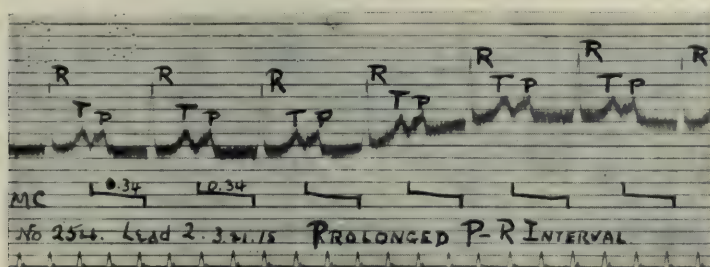


Fig. 4.—Case 1. Electrocardiogram (Lead 2) of M. C., three days later after the administration of 1 mg. of atropin. The slightly slower rate (longer diastole) and shorter P-R interval allow the P wave to be distinguished from the preceding T.

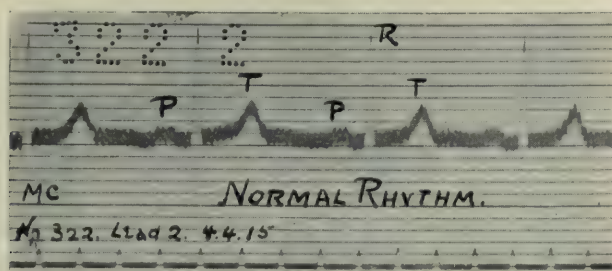


Fig. 5.—Case 1. Electrocardiogram (Lead 2) of M. C., showing normal rhythm and normal P-R interval.

of atropin medication (0.3 mg. hypodermically three times a day), the rhythm remained regular for several days; the P-R interval was not as much prolonged as before, and the P wave no longer coincided with the preceding T. In other words, although the block was not due to medication, and from the nature of the disease process was presumably due to an acute myocarditis involving His' bundle, nevertheless the administration of atropin was sufficient to prevent any beats being "dropped." Does this indicate that the vagus as well as the diseased bundle was a factor in the block, or does it indicate that atropin exerts a direct dromotropic effect on His' bundle? Two days after this tracing was taken, the acute arthritis recurred in one finger, together with fever and episcleritis of the right eye. Electrocardiograms at this period showed the same condition of partial block as before described. Atropin medication was again

begun, together with atophan. In four days the temperature had returned almost to normal, the patient was without pain, and the cardiac rhythm was regular. From that time until the patient's discharge from the hospital, electrocardiograms showed not only a regular rhythm, but a normal P-R interval (0.19). A slight recurrence of pain, swelling and redness in two knuckles of the left hand, with a rise of 1 degree in temperature, did not have any effect on the cardiac rhythm, and the patient progressed to an uninterrupted recovery from the acute rheumatism. It was later ascertained, however, that the patient died, within two months of the time of her discharge, of acute yellow atrophy of the liver. In the terminal illness the heart continued regular at the rate of 100 beats per minute. Necropsy was refused.

#### DISCUSSION

During an attack of recurrent acute articular rheumatism in a patient suffering with chronic mitral endocarditis, cardiac hypertrophy and probably some chronic myocarditis, there was presumably superimposed an acute myocardial involvement of His' bundle. This was extensive enough to lower the conductivity of the bundle sufficiently to cause partial heart block. Vagus stimulation failed to change the stage of heart block, but did slightly change its character. After the administration of atropin, impulse conduction was delayed but no longer blocked. Coincident with improvement in the other rheumatic symptoms, the block disappeared, to reappear again with a recrudescence of symptoms. At this time, during the administration of atropin, the dropped beats disappeared and the P-R interval became normal. A third minor recurrence of rheumatic symptoms, however, failed to affect the now normal heart rhythm, which continued normal as long as the patient was under observation. The administration of salicylates cannot be considered to have had any connection with the production of the block; not only because this class of drugs is not considered to have any effect on conductivity, but also because the degree of block in this case usually varied inversely with the degree of salicylic medication.

It is of interest that although the block was presumably of myocardial origin, it was nevertheless influenced by factors affecting the vagus, and thus indirectly affecting the damaged conductive system.

*II. Transient Complete A-V Block Due to Digitalis.*—Transient digitalis block of lesser degrees is not an uncommon condition, but a transient complete block, especially when following relatively small doses of digitalis, must be considered as very unusual.

CASE 2.—M. F., married, Irish, hospital orderly, aged 65, had been suffering for over a year with attacks of giddiness, weakness, loss of vision, and on two or more occasions apoplectiform attacks, which lasted some hours, but were not followed by any hemiplegia. On one of these occasions he was admitted to this hospital for one week, but no bradycardia or arrhythmia was observed, although tincture digitalis (0.32 c.c. three times a day) was given for six days. His past and family histories are unimportant, except for a life of hard work and exposure, with moderate use of alcohol, tobacco, tea and coffee.



*Physical Examination and Course.*—On the present admission, Jan. 11, 1916, the following pertinent signs were observed: blood pressure, systolic, 130, diastolic, 70; marked pyorrhea; emphysematous chest; cardiac dulness: right border 4 cm. from midline, left border 14.5 cm. from midline, outside of left mid-clavicular line; apex beat neither visible nor palpable; heart sounds weak, no murmurs; blood and urine normal.

Tincture of digitalis (0.65 c.c. three times a day) was given for two days, when it was noticed that the patient's pulse had become slow (38 beats per minute) and irregular. It was afterward ascertained that previous to admission, the patient had taken 0.3 c.c. of the tincture of digitalis four times a day for four days, thus making a total dosage of only 8.7 c.c. of the tincture spread over six days. An electrocardiogram taken at this time showed a complete block with

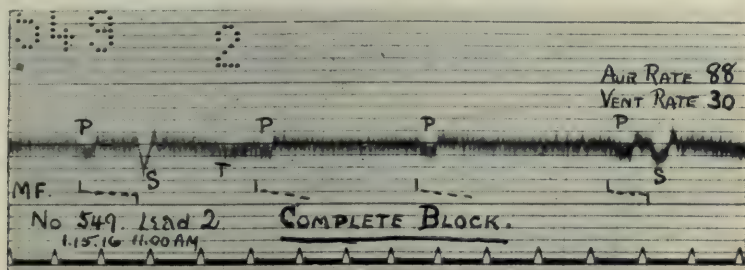


Fig. 6.—Case 2. Electrocardiogram (Lead 2) of M. F., showing complete heart block. Note that there is no constant relation between the occurrence of P and S (ventricular complex); although P twice falls before S, the P-R interval is different in both cases. The complete dissociation is even more obvious when the whole length of film is consulted. Note that the P waves are inverted and the ventricular complex of peculiar shape.

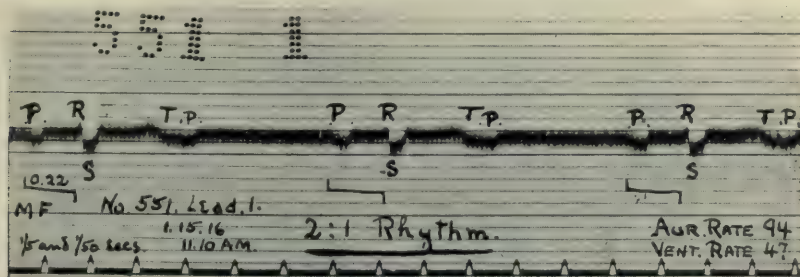


Fig. 7.—Case 2. Electrocardiogram (Lead 1) of M. F., showing partial heart block (2:1 rhythm) after the administration of 2 mg. of atropin.

a varying form of ventricular complex (auricular rate, 88; ventricular rate, 30). Not only were the ventricular deflections very small, but the heart sounds were quite inaudible. Immediately after this record was made, 2 mg. atropin were given hypodermically. The pulse rate rose in ten minutes to 50, and an electrocardiogram showed that the complete block had been replaced by a 2:1 rhythm.<sup>6</sup> The P-R interval was longer than normal (0.22 second). One-half hour later,

6. This might be interpreted as a normal rhythm, the second P wave being considered as part of a long diphasic T; but such an interpretation is highly improbable, as it would presuppose not only a very unusual form of T wave, but also the very slow auricular rate of 47.



complete block was reestablished (auricular rate, 100; ventricular, 46). During all this period the patient was quite comfortable, resting quietly in bed. Digitalis medication was stopped and thirty-six hours later the pulse rate had risen to 70, about which point it stayed during the rest of the patient's stay in the hospital. The P-R interval, however, remained prolonged (0.20 second), and was not changed by increasing the heart rate with atropin. When seen two months later, the patient had had no more "attacks," though he was still somewhat "shaky" in his legs. Electrocardiogram showed a normal rhythm, but the P-R interval still remained long.

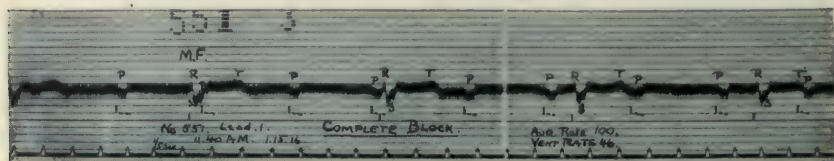


Fig. 8.—Case 2. Electrocardiogram (Lead 1) of M. F., showing the return to complete heart block, forty minutes after the administration of atropin. Note that P occurs at regular intervals but without relation to, and occasionally superimposed on, the ventricular complex (R S).

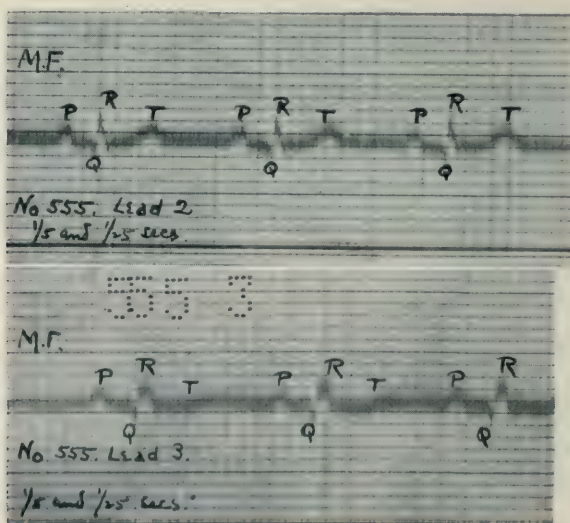
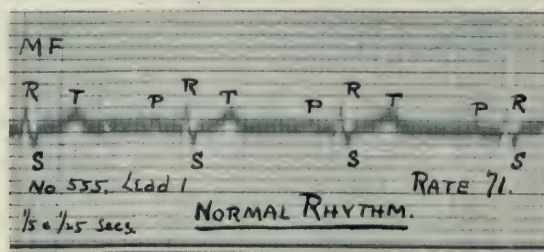


Fig. 9.—Case 2. Electrocardiogram of M. F. (three leads). Normal rhythm. Note also that the P waves are no longer inverted and that the ventricular complexes are of normal shape.

## DISCUSSION

In an old man suffering from chronic myocardial degeneration and arteriosclerosis, and subject to spells of weakness and unconsciousness, moderate digitalis dosage for less than a week, brought on a transient, complete A-V heart block, with varying types of ventricular complexes. This was temporarily relieved by atropin, and disappeared spontaneously thirty-six hours after the digitalis was stopped. Although digitalis was obviously the determining factor in the causation of the block, it is but fair to assume that degenerative changes in the fibers of His' bundle contributed to the easier production of the digitalis effect. The temporary cessation of the block after the administration of atropin offers an interesting comparison with the first case. If in that case, as seems probable, atropin exerted a direct dromotropic effect on His' bundle, one might assume that here also the atropin effect may have been obtained partly through direct action on the bundle, although on account of the obvious importance of digitalis effects, the paralyzing action on the vagus must, of course, also be taken into account. The varying forms of ventricular complexes during the digitalis period are of the same kind as previously described by Cohn,<sup>7</sup> Oppenheimer and Williams<sup>8</sup> and Christian.<sup>9</sup>

*III. Development of Defective Conductivity of Right Branch of His' Bundle.*—The subject of defective conductivity in one or other branch of His' bundle has been thoroughly presented by Carter<sup>10</sup> and Matthewson<sup>11</sup> from the clinical, and by Eppinger and Rothberger<sup>12</sup> from the experimental, side. At least one case is on record also in which transient block occurred in the right branch of His' bundle during a febrile attack (Lewis<sup>13</sup>), and Carter cites instances in which, as in the present case, the bundle branch block is complicated by extrasystoles. The following case, however, is unique in one particular,

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7. Cohn, A. E.: A Case of Transient Complete Auriculo-Ventricular Dissociation, Showing Constantly Varying Ventricular Complexes, *Heart*, 1913, **5**, 5.

8. Oppenheimer, B. S., and Williams, H. B.: Prolonged Complete Heart Block without Lesion of the Bundle of His and with Frequent Changes in the Idioventricular Electrical Complexes, *Proc. Soc. Exper. Biol. and Med.*, 1913, **10**, 86.

9. Christian, H. A.: Transient Auriculoventricular Dissociation with Varying Ventricular Complexes Caused by Digitalis, *THE ARCHIVES INT. MED.*, 1915, **16**, 341.

10. Carter, E. P.: Clinical Observations on Defective Conduction in the Branches of the Ventricular Bundle, *THE ARCHIVES INT. MED.*, 1914, **13**, 803.

11. Matthewson, G. D.: Lesions of the Branches of the A-V Bundle, *Heart*, 1913, **4**, 385.

12. Eppinger, H., and Rothberger: Ueber die Folgen der Durchschneidung der Tawaraschen Schenkel des Reizleitungssystem, *Ztschr. f. klin. Med.*, 1910, **70**, 1.

13. Lewis, T.: Certain Physical Signs of Myocardial Involvement, *Brit. Med. Jour.*, 1913, **1**, 484.



that the earliest record was taken at the time when the branch block was apparently in the process of formation, and opportunity was offered to follow the case until the block was permanently established.<sup>14</sup>

CASE 3.—F. L., man, married, retired, aged 76, had been under observation for ten years for symptoms suggesting arteriosclerosis and myocardial weakness (precordial pains, especially after meals, dyspnea on exertion, enlarged liver, occasional cough). After exercise the precordial oppression increased and the pain occasionally radiated down either arm. The pulse had always been slow (55 to 65), the blood pressure was but moderately increased (average examples are: in 1912, systolic, 130; diastolic, 80; in 1913, 135 and 90; 1914, 155 and 80; 1915, 155 and 80; 1916, 155 and 70). A systolic murmur and an occasional slight arrhythmia had been noticed in the previous two years. The heart was enlarged to the left, the supracardiac dulness increased and the lungs emphysematous. The blood and urine were negative.

Except for an attack of biliary obstruction twenty-five years previously, and a tendency to constipation and flatulence, the past medical history is negative. For years the patient had smoked six to twelve cigars a day and indulged moderately in whisky. No venereal disease. Family history negative.

One month after the last electrocardiogram was taken, the patient died suddenly of acute cardiac failure. No necropsy was had.

*Examination and Course.*—In the first electrocardiogram, taken in January, 1915, although most of the complexes are of the type indicating left ventricular preponderance, occasionally one appears with the characteristic form of defective branch conduction, that is, notching and prolongation of the Q-R-S interval to more than 0.1 second. These occur as premature contractions. (The electrocardiogram is further complicated by the appearance of occasional auricular extrasystoles.) One month later almost all complexes were of this form and present typical examples of defective conductivity of the right branch of His' bundle. They are no longer premature. The auricular extrasystoles were still present. The P-R interval also had been prolonged from 0.20 second to 0.25 second. As the patient had been taking moderate doses of digitalis during this month, it was thought that a digitalis block might have been present, but 2 mg. of atropin hypodermically only raised the rate from 63 to 105 without altering the block. It became impossible to make further studies at this time, as the patient was living in another city. One year later, however, he returned, having been given digitalis for one month previously, and again the branch defect was found, this time in every complex. In fact, the electrocardiogram taken at this time was almost the counterpart of the one taken the year before, as far as the blocked complexes were concerned. All digitalis medication was stopped, but electrocardiograms taken on several occasions after this, on one occasion after one week's administration of atropin ( $\frac{1}{150}$  grain by mouth four times a day) showed the same condition of defective conductivity to be constantly present. The patient felt badly when digitalis was given and was subjectively improved during the administration of atropin.

#### DISCUSSION

A man 76 years of age, suffering from arteriosclerosis, cardiac hypertrophy and chronic myocarditis, and mild anginoid attacks, was

14. Oppenheimer, B. S., and Rothschild, M. H.: Abnormalities in the Q R S Groups of the Electrocardiogram Associated with Myocardial Involvement. Soc. Exper. Biol. and Med., Dec. 20, 1916. These authors have pointed out that electrocardiograms can be produced by lesions involving terminal fibers of a branch of His' bundle, and claim a different picture for a block of the main stem of a branch.



examined electrocardiographically to determine the form of arrhythmia that was present. This was found to be due to occasional auricular extrasystoles, and anomalous ventricular beats, due to deficient conductivity in the right branch of His' bundle. One month later, almost all complexes were of this type, but no longer premature. The P-R interval was prolonged to 0.25 second, and the condition was not altered

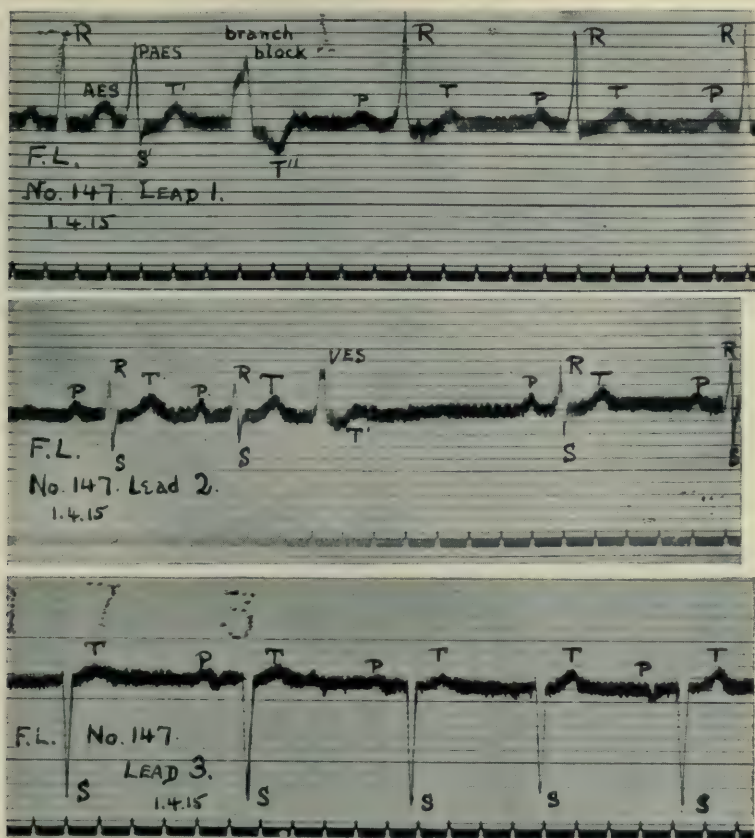


Fig. 10.—Case 3. Electrocardiogram of F. L. (three leads). Note that although most of the complexes are of the type indicating preponderance of the left ventricle, one in Lead 1 occurring prematurely is notched and broader (that is, slower). In the light of future records this is undoubtedly due to the impulse being blocked in the right branch of His' bundle. It is preceded by an auricular extrasystole and in Lead 2, an isolated ventricular extrasystole (probably of the branch block type) occurs. For purposes of reproduction, this print and the Q R S group of a few others have been retouched.

by atropin. One year later, in spite of long continued abstinence from digitalis, the same condition of defective conductivity was found to be present. It is, therefore, safe to assume that the defective conductivity (probably caused by the chronic myocarditis) was developing when

the patient was first seen and later became permanent, and it is important to note that the block developed without clinical symptoms or signs other than those revealed by the string galvanometer.

*IV. Transient Prolongation of P-R Interval (of Unexplained Origin), Associated with Paroxysmal Tachycardia.*—Prolongation of the P-R interval as a result of digitalis medication is too common a condition to require further comment. In the present instance, however, in a healthy young adult who also had attacks of paroxysmal tachycardia, the condition had occurred on at least two occasions in the absence of digitalis medication, or in fact of any other adequate cause.

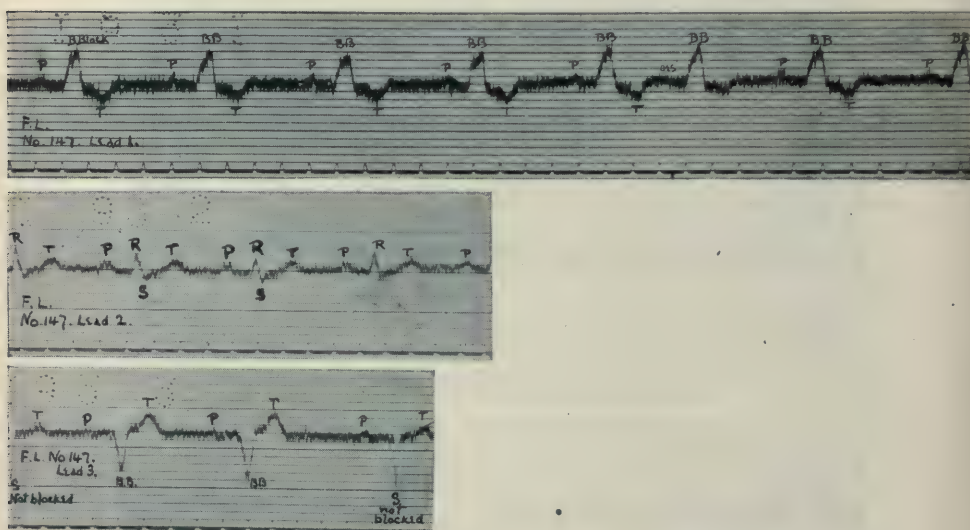


Fig. 11.—Case 3. Electrocardiogram of F. L. (three leads), showing defective conductivity (block in right branch of His' bundle) in almost all leads. Note that in Lead 3, two beats (S) are of the original type; all the others are notched and slow. An auricular extrasystole in Lead 1 is followed by the same kind of complex.

**CASE 4.**—H. W. B., medical student, aged 23, was admitted to the student's ward of the University Hospital, Feb. 2, 1915, suffering from an attack of acute tonsillitis, vague joint pains and very rapid heart rate (over 150). These conditions subsided, after one day's rest in bed, with such suddenness that a diagnosis of paroxysmal tachycardia was made. After the paroxysm the pulse varied between 72 and 100.

The past history showed that the patient had had a slight attack of rheumatic fever when 10 years old, had had frequent attacks of tonsillitis since that time, and had a mild, chronic, atrophic nasopharyngitis. He smoked four or five cigarets and drank two cups of coffee a day, but took no alcohol. He denied any history of venereal disease. He was distinctly neurotic, with vasomotor instability, and at the time of admission had been studying unusually hard and worrying over examinations. His health was otherwise excellent

and he could undertake violent exercise without any cardiac distress, further than that he "notices that he has a heart."

*Examination and Course.*—The first electrocardiographic examination taken the day after admission, when the patient was feeling well, revealed a regular slow rhythm, normal in every respect except for the greatly prolonged P-R interval (0.36 second). On account of the recent history of tachycardia, however, another record was taken immediately after exercise. After climbing two flights of stairs, a task sufficient to raise a normal heart rate from 72 to 96, the patient's heart rate was raised from 78 to 108. An electrocardiogram

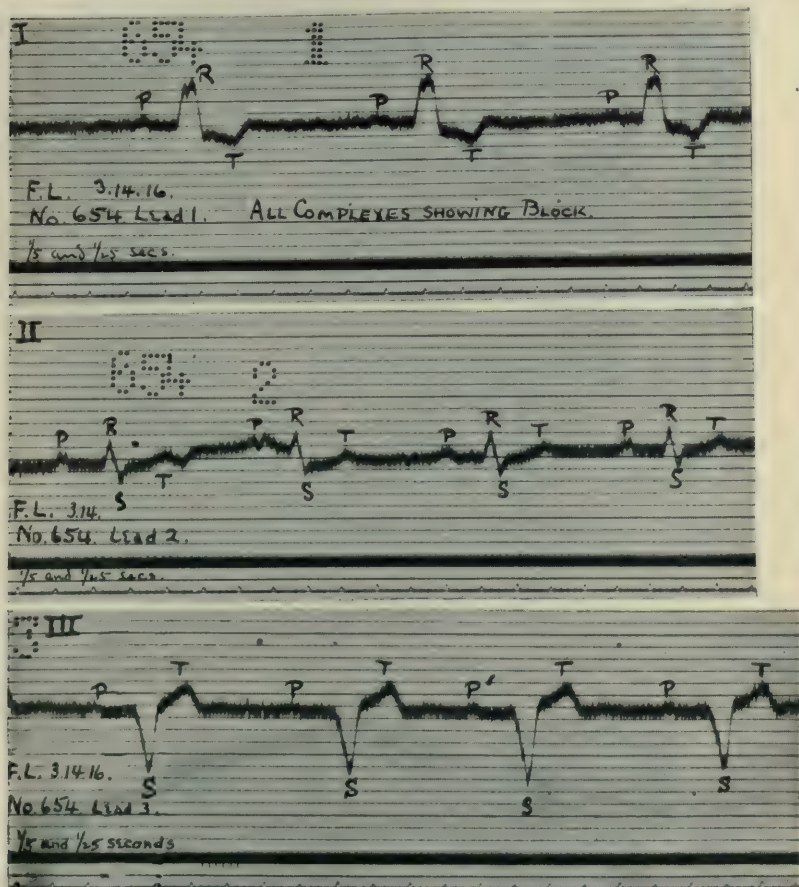


Fig. 12.—Case 3. Electrocardiogram of F. L. (three leads), showing defective conductivity (block of right branch of His' bundle) constant in all complexes.

taken at this time showed disappearance of the P wave (fused with the preceding T) during the rapid period (chiefly due to the prolonged P-R interval), and fortunately also caught the sudden cessation of the paroxysm, with reappearance of P. (Change in rate from 100 to 67.) (This record (Fig. 13) is almost a duplicate of figure 62 in Lewis' Clinical Electrocardiography). A record was then taken during forced respiration, which produced an arrhythmia, due both to sinus arrhythmia and to changes in the P-R interval. Atropin



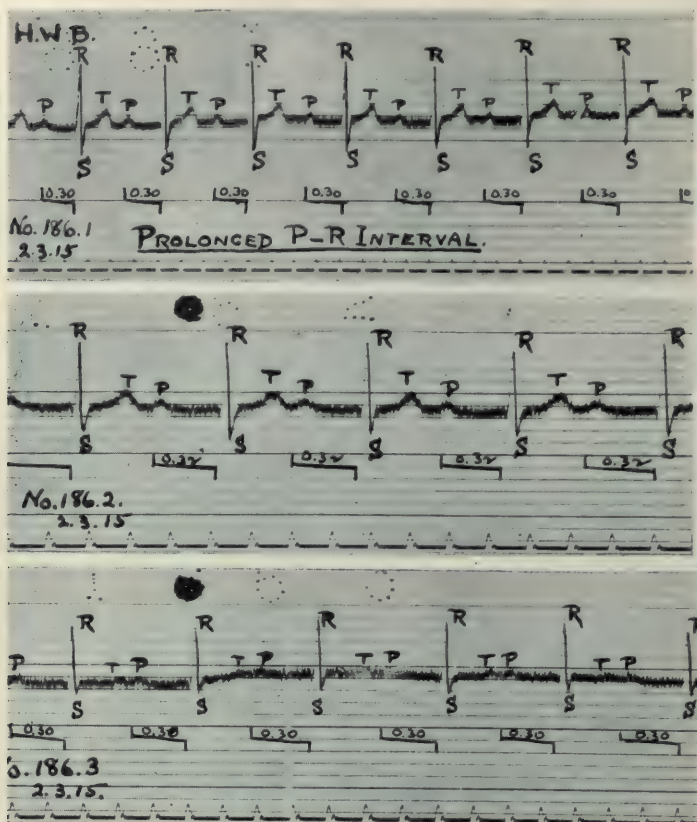


Fig. 13.—Case 4. Electrocardiogram of H. W. B. (three leads), showing prolongation of the P-R interval, earliest stage of heart block. Note that except for the greatly prolonged P-R interval (0.30 second) the record is normal.

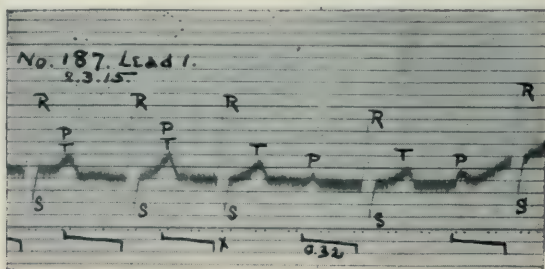


Fig. 14.—Case 4. Electrocardiogram of H. W. B. (Lead 1), showing termination of an attack of paroxysmal tachycardia. The P wave during the paroxysm is superimposed on the preceding T, so that it is apparent that the P-R interval remains prolonged during the paroxysmal period. The fact that this combined wave is higher than the single T wave of the normal period is an indication that the P waves of the paroxysm were upright, and therefore arose at or near the sinus.

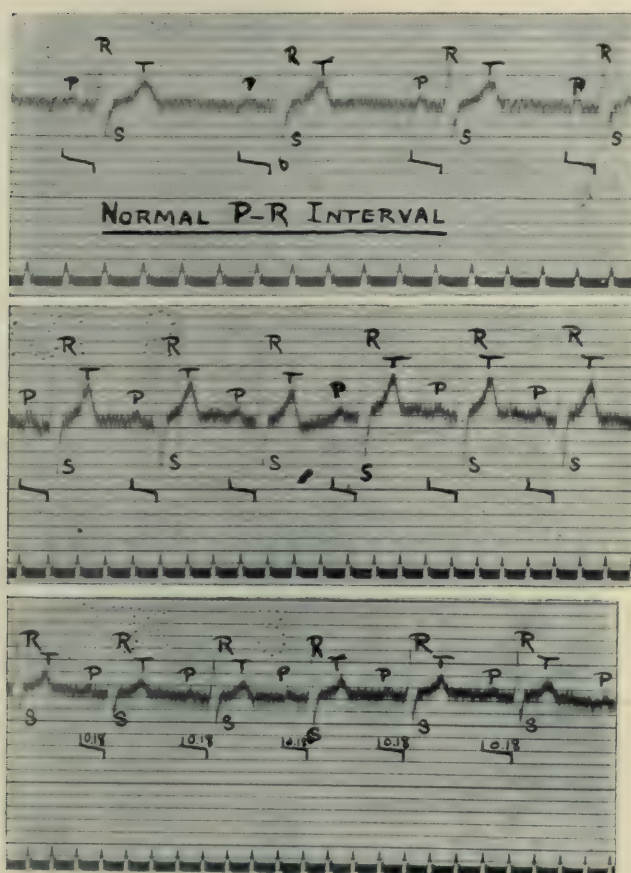


Fig. 15.—Case 4. Electrocardiogram of H. W. B. (three leads) showing normal rhythm with normal P-R interval (0.18 second).

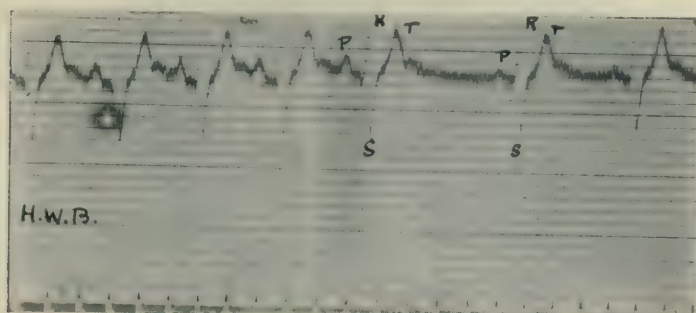


Fig. 16.—Case 4. Electrocardiogram of H. W. B. (Lead 2), showing termination of an attack of paroxysmal tachycardia with retention of the normal P-R interval. Note that the P wave is upright throughout.

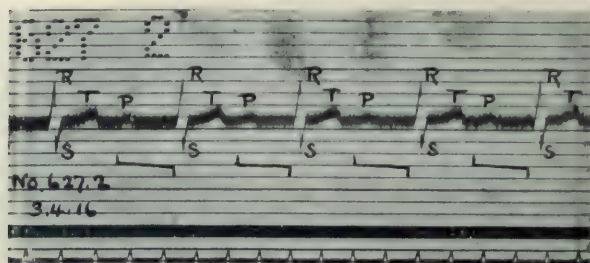


Fig. 17.—Electrocardiogram of H. W. B. (three leads), showing prolongation of the P-R interval one year later (similar to Fig. 12).

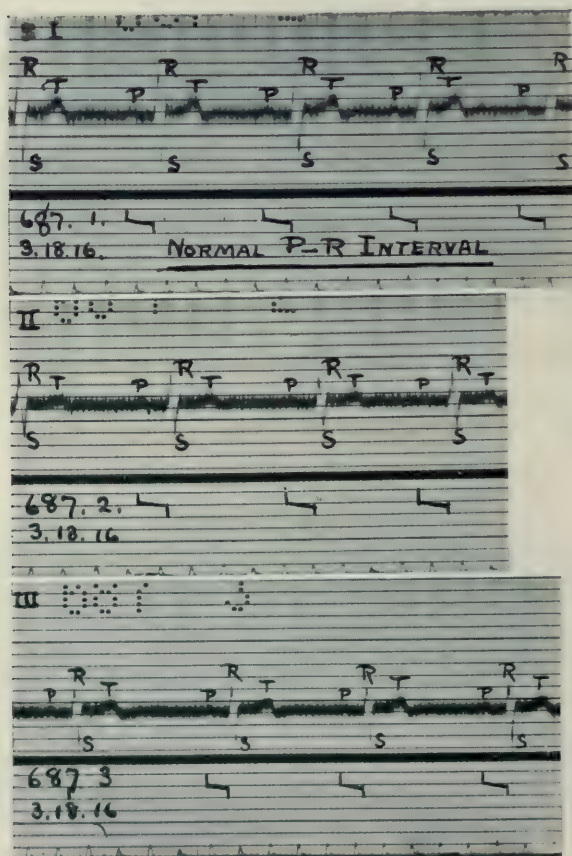


Fig. 18.—Electrocardiogram of H. W. B. (three leads), showing normal P-R interval, two weeks later than Figure 17 (similar to Fig. 14).



(2 mg. hypodermically) produced a tachycardia similar to that produced by exercise, raising the pulse rate from 85 to 128.

One month later, no medicine having been taken since leaving the hospital, an electrocardiogram showed that the P-R interval was now within normal limits (0.18 second). Except for an accentuation of S and T in all leads, the record is otherwise the same as on the previous examination. As the prolonged P-R interval produced no other signs or symptoms, the patient was ignorant of when the change to normal rhythm occurred. Exercise, however, produced a tachycardia similar to that first observed, except that as the P-R interval remained normal P and T were no longer fused. The rapid heart rate slowed to a normal rate during one cycle, as on the previous occasion. The post paroxysmal pause is longer than that of normal cycles, indicating that the P waves of the paroxysm are ectopic.

The patient was not seen for a year, during which time he was in good health. Two days before this visit, for no apparent cause (except a slight increase in cigaret smoking and in the chronic throat trouble), he observed that his pulse rate was 120, and on getting up suddenly, noticed that he would "feel his heart." Electrocardiograms again showed a long P-R interval (0.32 second), with excessive increase in the heart rate after exercise, and fusion of the P and T waves. This persisted for at least a week; but three weeks later, after the cigaret smoking had been stopped and the throat condition had improved, the P-R interval had again become normal (0.18 second). Since then a year has elapsed and the patient has continued in good health, without any cardiac symptoms.

#### DISCUSSION

A young, neurotic, male adult, with subjective cardiac symptoms only, was observed on different occasions to have an unusually long P-R interval and a tendency to the auricular (or possibly a sinus) form of paroxysmal tachycardia. This latter occurred at least twice spontaneously, and could be produced at will by exercise or with atropin. It occurred independently of the state of conductivity of the A-V system. For no apparent adequate cause, the P-R interval had been observed twice (an interval of over a year elapsing) to be more than 0.3 second. Once this followed a period of hard study and worry, and once an increase in cigaret smoking in the presence of a chronically inflamed throat. On both occasions the P-R interval returned to normal within a few weeks of the cessation of these conditions, and yet it would be presumptuous to assume that they had a causal relationship.

#### GENERAL SUMMARY

1. Four different types of transient heart block are described, analyzed and discussed:

(a) Transient partial A-V block of myocardial origin, occurring during an exacerbation of acute rheumatic carditis, varying with the degree of arthritis, yet responding to atropin.

(b) Transient complete A-V block, brought on by digitalis, and temporarily reducible by atropin to a 2:1 rhythm.

(c) The development of defective conductivity in the right branch

of His' bundle in an old man suffering with arteriosclerosis, chronic myocarditis and anginoid symptoms.

(*d*) Transient periods of prolongation of the P-R interval (to more than 0.3 second) without adequate cause in a healthy young adult male. He is also subject to paroxysmal tachycardia of auricular (or sinus) origin, which occurs independently of and does not affect the state of the conductive system.

# THE CARBON DIOXID CONTENT OF BLOOD AND OF ALVEOLAR AIR IN OBSTRUCTED EXPIRATION \*

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NEW YORK

In asthma the following clinical observations have been made:

1. Low pulse pressure in those cases uncomplicated by arteriosclerosis, probably due to diminished systolic volume.
2. Loud pulmonic second sound pointing towards increased resistance in the lesser circulation.
3. Polycythemia, possibly a teleologic phenomenon designed to compensate for a lessened minute volume output from the left ventricle.
4. Enlarged veins in the neck and cyanosis suggesting a certain amount of general venous stasis.

These clinical facts led us to suspect that circulatory disturbances might account in large part for the conditions associated with obstructed expiration. On account of the lack of adequate clinical material, we decided to study this subject in the experimental animal. Dogs were used.

## TECHNIC

The animals received a preliminary injection of 0.16 c.c. of a 2 per cent. solution of morphin per kilogram of body weight. Full anesthesia was produced by the administration through a stomach tube of a saturated solution of chlorbutanol (chloretone) (15 c.c. per kilogram of body weight.)

Tracheotomy was performed. A T-tube was inserted into the trachea and it was so arranged that there was no increase in dead space.<sup>1</sup> As soon as an even anesthesia was obtained, the expired air was collected in a Dreser tube and the minute volume was determined. The carbon dioxide in the blood was determined by means of the Barcroft-Haldane<sup>2</sup> method, that in the alveolar air by Henderson's<sup>3</sup> method. Samples of air were obtained by forcible compression of the thorax at the height of inspiration. A careful series of controls indicated that under the conditions of the experiment, with unobstructed expiration, the CO<sub>2</sub> content of the blood and the alveolar air varied with the minute volume, thus corroborating the work of Haldane and Priestley.<sup>4</sup> Anesthesia for varying lengths of time produced effects varying only with the ventilation.

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\* From the Laboratory of Physiology, University and Bellevue Hospital Medical College.

\* This work was done during tenure of the C. A. Herter Fellowship in Research Medicine.

1. After our manuscript had been completed, we noted the work of Henderson, Chillingworth and Whitney and that of J. S. Haldane (*Am. Jour. Physiol.*, 1915, **38**, 1) on the variations in dead space. These changes, however, take place only with greatly increased ventilation and do not affect our experiments.

2. Barcroft and Haldane: *Jour. Physiol.*, 1902, **28**, 232.

3. Henderson: *Am. Jour. Physiol.*, 1911-1912, **29**, 441.

4. Haldane and Priestley: *Jour. Physiol.*, 1904-1905, **32**, 225.



In another series, a one-way valve was attached to the vertical end of the T-tube, and by means of a screw, varying degrees of obstruction to expiration were produced. This was our method of simulating the asthmatic attack. The anatomic findings show that we have been successful, especially with regard to the morphologic picture.<sup>6</sup> The obstruction lasted for varying lengths of time. Control determinations were always made before the valve was inserted,

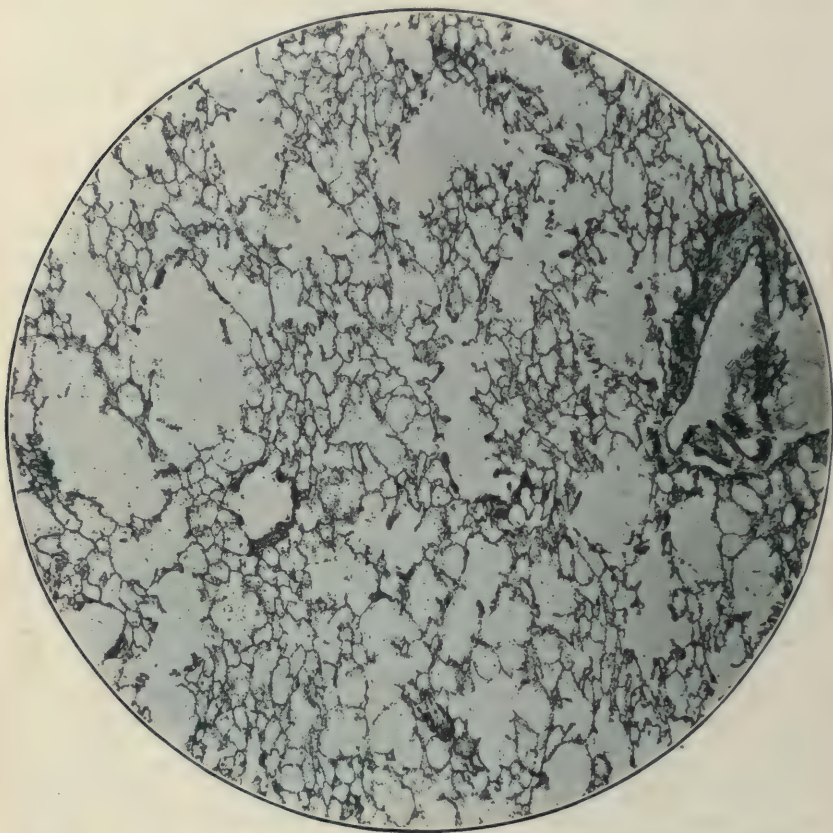


Fig. 1.—Section of lung from valve dog which shows dilated infundibula and ruptured alveoli. Note the extremely thin walls of the alveoli and the ruptured ends projecting into the lumen of the infundibulum. There is no area where normal lung structure could be demonstrated.

5. A pathologic study was made of the lungs of our valve dogs by Dr. Alexander Fraser of the Department of Pathology of University and Bellevue Hospital Medical College, to whom we here express our sincere thanks. He found the macroscopic and microscopic evidences of emphysema with the exception of increase in connective tissue. There were also areas resembling infarctions, probably due to rhexis as a result of the increased resistance in the pulmonary circuit. One of Hoover's patients also coughed up, during an asthmatic attack, a frothy, blood-stained sputum, the source of which might have been one of these areas mentioned by Dr. Fraser. It is of interest to add that Stahelin also noted the occurrence of hemoptysis in some cases.

or before expiration was obstructed by screwing down the valve. A marked increase in the  $\text{CO}_2$  content of the blood and the alveolar air occurred especially where increased ventilation had not occurred. In most instances this attempt at compensation showed itself by increased ventilation due to the sensitiveness of the respiratory center to slight rises in the  $\text{CO}_2$  pressure in the alveolar air. (Haldane and Priestley.<sup>4</sup>) Usually, however, this attempt at compensation was inadequate, because "there seems to be an optimum of respiratory volume beyond which the effectiveness of ventilation is not proportional to the increase in exchange." (Hoover.<sup>6</sup>)

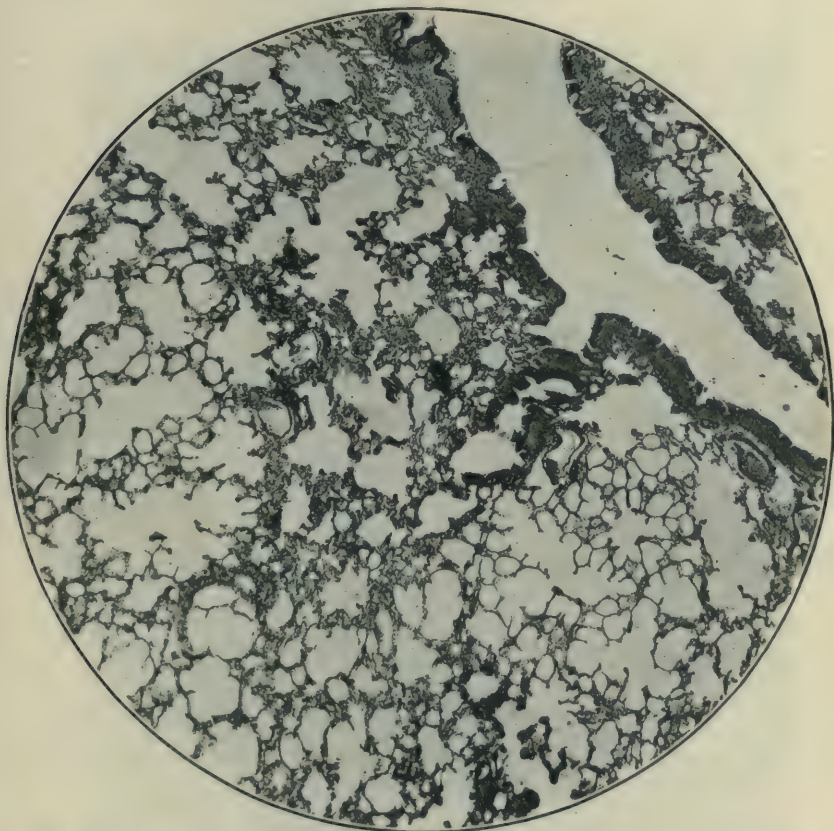


Fig. 2.—Section of lung from normal dog which shows a well distended bit of lung<sup>7</sup> which did not show signs of emphysema macroscopically. The infundibula are fully distended, but the normal relationship between infundibula and alveoli is preserved. The dark areas represent round-cell infiltrations and red cells.

As soon as the obstruction was introduced, the type of respiration changed. Expiration became prolonged, the rate slowed, and the abdominal wall muscles also aided in the attempt to force air out. It seemed possible that increased muscular work might be a factor tending to raise the  $\text{CO}_2$  content of the

6. Hoover: *THE ARCHIVES INT. MED.*, 1913, **11**, 52.

TABLE 1.—CONTROL EXPERIMENTS

Experiment	Minute Volume	Blood CO <sub>2</sub> Volume, per Cent.	Alveolar Air CO <sub>2</sub> Volume per Cent.	Duration of Experiment, Hours
1 Begin.....	1,800	52.86	5.34	6
End.....	2,000	55.53	5.14	
2 Begin.....	1,300	50.59	5.76	7
End.....	900	61.18	6.11	
3 Begin.....	2,700	48.11	5.62	6
End.....	1,200	80.21	8.53	
4 Begin.....	2,250	62.10	5.11	6
End.....	4,400	48.58	4.53	
5 Begin.....	1,120	60.41	5.28	8
End.....	1,710	45.74	5.01	
6 Begin.....	2,870	48.44	7.15	6
End.....	1,540	64.61	8.28	
7 Begin.....	1,400	60.22	7.33	6
End.....	720	69.20	8.19	
8 Begin.....	2,700	46.46	5.26	6
End.....	1,500	51.02	6.25	

TABLE 2.—VALVE EXPERIMENTS

Experiment	Minute Volume	Blood CO <sub>2</sub> Volume, per Cent.	Alveolar Air CO <sub>2</sub> Volume per Cent.	Duration of Experiment, Hours
9 Begin.....	1,560	53.23	5.66	3
End.....	2,160	61.82	6.88	
10 Begin.....	1,620	50.80	4.92	8
End.....	3,625	69.88	5.43	
11 Begin.....	1,200	35.20	4.01	6
End.....	2,400	47.80	4.37	
12 Begin.....	1,800	44.74	5.12	8
End.....	3,000	64.20	6.35	
13 Begin.....	1,500	50.68	6.07	6
End.....	1,600	81.40	9.22	
14 Begin.....	1,200	44.53	4.64	6
End.....	1,980	59.43	6.30	

blood.<sup>7</sup> A series of controls was carried out with strychninized dogs. The animals received 2 c.c. of a 1 to 1,000 strychnin solution, hypodermatically, in divided doses, at five minute intervals until a convulsive response was produced by tapping on the nose. These results agreed with those of our control animals, in which the CO<sub>2</sub> in the blood and in the alveolar air varied according to the ventilation.

7. It is of interest in this connection to note the work of Wilson, Stearns and Thurlow (Jour. Biol. Chem., 1915, **23**, 89) who found that tetany following parathyroidectomy tends to lessen the alkalinity and to lower the CO<sub>2</sub> tension of the blood. Our experiments with strychninized animals tend to confirm these results.



TABLE 3.—STRYCHNIN EXPERIMENTS

Experiment	Minute Volume	Blood CO <sub>2</sub> Volume, per Cent.	Alveolar Air CO <sub>2</sub> Volume per Cent.	Duration of Experiment, Hours
15 Begin.....	1,080	58.44	7.31	4
End.....	1,440	44.51	6.66	
16 Begin.....	1,800	51.66	5.89	4
End.....	2,100	52.35	5.95	

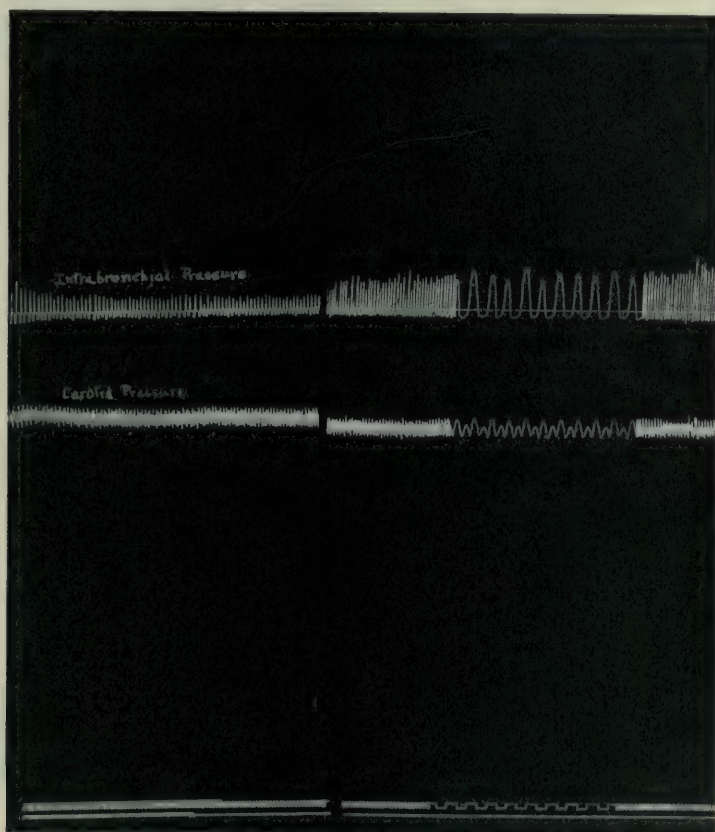


Fig. 3.—Period of beginning obstruction. Note the ratio between the duration of positive and negative intrabronchial pressure. Ratio, negative 1: positive 1.

To get some insight as to events occurring in the circulatory system, carotid pressure observations were made simultaneously with the determination of the intrabronchial pressure.

From a study of the tracings we can conclude that there is at the beginning of expiration a preliminary squeezing out of blood from the pulmonary capillaries and veins into the left heart. This increases the

systolic output from the left ventricle and the carotid pressure rises for a few seconds. After a few beats, however, there is a fall in blood pressure, due to the fact that the intrabronchial pressure exceeds the capillary pressure and interferes with the flow through the compressed capillaries, thus diminishing the return of blood to the left auricle. Suddenly, at the beginning of inspiration, when the intrabronchial pressure falls, the drop in systolic pressure which began toward the

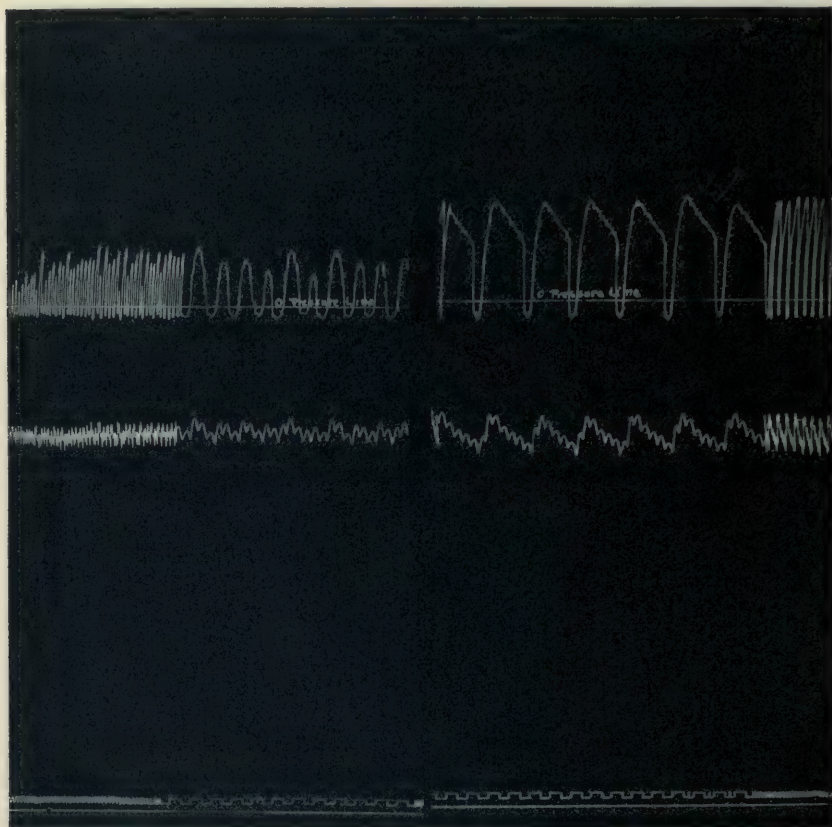


Fig. 4.—Period of increasing obstruction. Ratio of negative pressure to positive pressure. Tracing on left, negative 3: positive 5; tracing on right, negative 1: positive 6.

end of expiration is still further increased. The depleted pulmonary capillaries take up the blood from the right ventricle, and thereby lessen the flow to the left heart. There probably is a shutting off of free flow from the superior vena cava, but due to the rise in the intra-abdominal pressure, the inferior vena cava empties itself into the right heart and thus assures a definite supply of blood to the right heart.

The tracings indicate a diminution of systolic output with rising intra-bronchial pressure, and a change in the time relationship between inspiration and expiration. Whereas the normal ratio of inspiration to expiration is 5:4, in our animals it approximated 1:4. On the whole, the tracings point out clearly that there is a distinct interference with

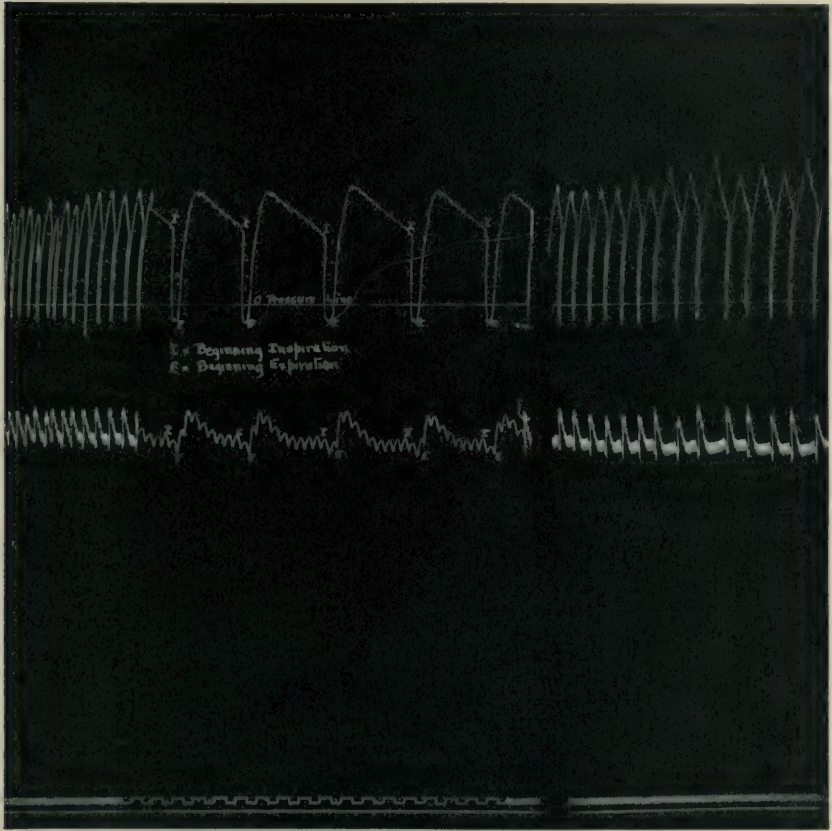


Fig. 5.—Period of increasing obstruction. Ratio of negative pressure to positive pressure. Negative 1: positive 7.

blood flow through the lungs, and therefore with proper aeration, Gerhardt,<sup>8</sup> Minkowski,<sup>9</sup> Tendeloo,<sup>10</sup> Stewart,<sup>11</sup> Romanoff,<sup>12</sup> Cloetta<sup>13</sup> and Bruns<sup>14</sup> are agreed that even slight rises in the intrabronchial

8. Gerhardt: *Ztschr. f. klin. Med.*, 1904, **55**, 195.

9. Minkowski: *Therap. d. Gegenw.*, 1912, **53**, New Series, **14**, 1.

10. Tendeloo: *Ergebn. d. inn. Med. u. Kinderh.*, 1910, **6**, 1.

11. Stewart: *Jour. Physiol.*, 1894, No. 15, p. 31.

12. Romanoff: *Arch. f. exper. Path. u. Pharmacol.*, 1910-1911, **64**, 183.

13. Cloetta: *Arch. f. exper. Path. u. Pharmacol.*, 1911, **66**, 409.

14. Bruns: *Deutsch. Arch. f. klin. Med.*, 1912, **108**, 469.



pressure cause considerable obstruction to blood flow through the lungs, the pressure in the pulmonary capillaries not being much above 0.

Hoover<sup>15</sup> has attempted to explain the insufficient aeration of the blood in asthmatics on a respiratory basis. He found that the CO<sub>2</sub> content of the alveolar air rose in the asthmatic attack, especially where the patient had already suffered from emphysema. He first thought that an increase in the dead space, with impaired alveolar ventilation,

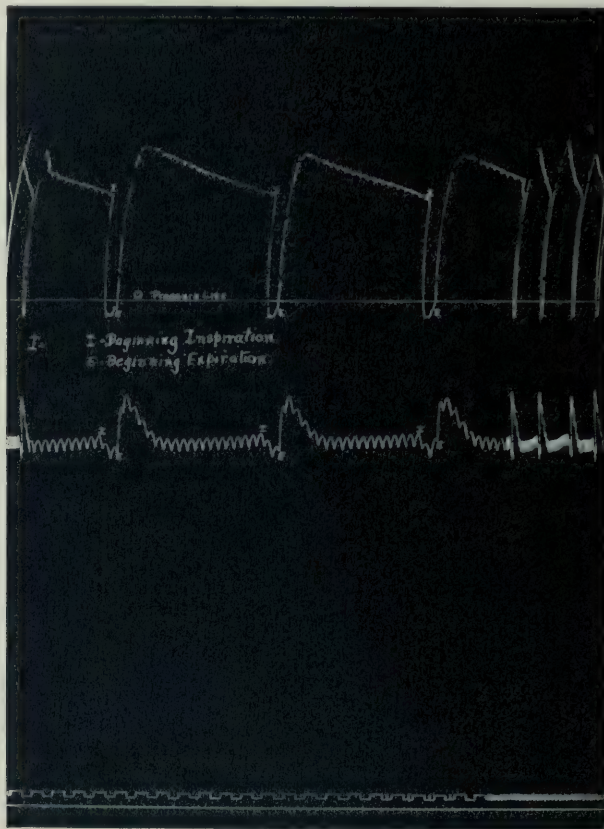


Fig. 6.—Period of maximum obstruction. Intrabronchial pressure, 22 to 26 mm. mercury. Ratio of negative pressure to positive pressure, negative 1; positive 15.

was the cause of this rise. Later observations showed that there was no appreciable increase in the dead space in these cases. In fact, he says that "the dead space is no larger in these cases than in normal persons." The cause of the disturbance in aeration could not be circulatory, for he says "to produce cyanosis by impairment in circulation,

15. Hoover: THE ARCHIVES INT. MED., 1915, **15**, 1 and 501.

the evidence of venous stasis must be very great." Yet we all know that most emphysematous patients show rather well marked cyanosis without any evidence of hepatic congestion, edema, etc. He concluded from his studies that "the real difficulty of ventilation in asthma lies in a distention of the infundibula, and this fails to permit an equal diffusion of  $\text{CO}_2$  throughout the alveolar air," an explanation, which does not seem plausible, especially when one recalls the relatively large diffusion coefficient for  $\text{CO}_2$ .

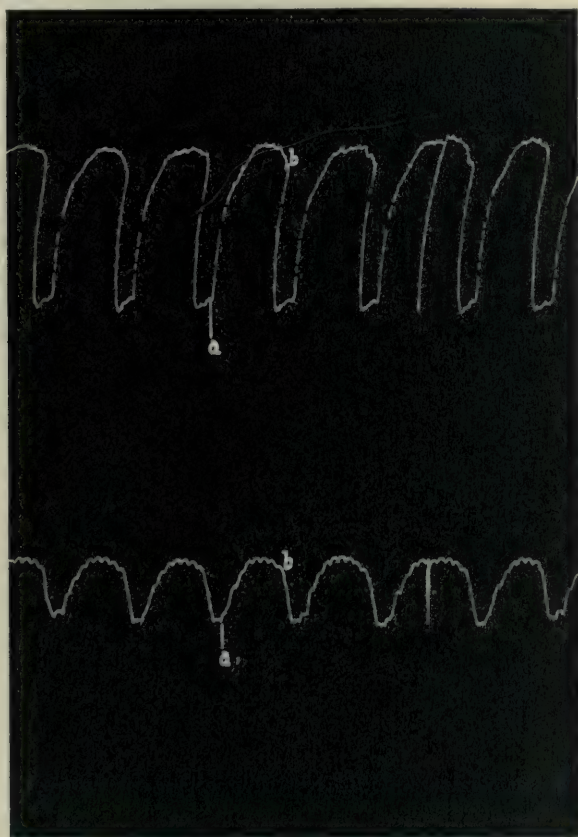


Fig. 7.—Note decreasing systolic volume as intrabronchial pressure rose. Upper tracing, intrabronchial pressure; lower tracing, carotid pressure; a, beginning of expiration, b, beginning of inspiration.

In view of all these facts, we are inclined to believe that the circulatory factor, contrary to Hoover, is the important one. The cough in asthmatics, which, according to Gerhardt, can raise the intrabronchial pressure to 57 mm. of mercury, and according to Aron to 90 mm. of mercury, would distinctly embarrass the lesser circulation as outlined

in the foregoing, and the voluntary attempts to aid expiration would accentuate the condition. An asthmatic attack in an emphysematous individual would still further distend an already overexpanded lung. Hoover found especially high readings for blood and alveolar air  $\text{CO}_2$  in such cases. If we take into account the time relationship between inspiration and expiration, we find that in our animals expiration lasted about four times as long as inspiration. There is, therefore, during four-fifths of the respiratory cycle, the long drawn-out expiration, a positive intrabronchial pressure as a result of which resistance to the flow of blood through the pulmonary capillaries is great. During this phase there is a little opportunity for gaseous exchange. Then there follows only a short inspiration, during which the  $\text{CO}_2$  is released as the blood is taken up by the depleted pulmonary sponge. Stahelin<sup>16</sup> remarks that during the Valsalva test, straining with a closed glottis, one sees during Roentgen-ray examination the heart decrease in size and the carotid pressure fall, an evidence of the fact that due to the rise in intrathoracic pressure, little blood can enter the heart, and it therefore pumps itself out. This is but an extreme degree of the mechanism we have described. So too, we are all familiar with the cyanosis which occurs during the pertussis spasm, the rise in intrathoracic pressure causing venous stasis and interfering with free flow to the heart. The absence of a permanent fall in blood pressure in our animals may be explained by a compensatory vasomotor contraction of the systemic arterioles and the emptying of the inferior vena cava by the increased intra-abdominal pressure.

#### SUMMARY

The high  $\text{CO}_2$  content of the alveolar air in asthma and obstructed expiration in general is due to a circulatory cause. The rise in intrabronchial pressure during the long expiration interferes with the free flow of blood through the pulmonary capillaries and causes a damming back of the blood on the venous side. There is a consequent accumulation of  $\text{CO}_2$  in the blood with the liberation of  $\text{CO}_2$  into the alveolar air, chiefly during the short inspiratory phase of asthmatic breathing.

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16. Stahelin: *Jahresk. f. ärztl. Fortbild.*, München., 1913, Part 2, p. 50.



# A STUDY OF BLOOD SUGAR

## A COMPARISON OF THE TOLERANCE FOR GLUCOSE IN DIABETIC AND NORMAL SUBJECTS \*

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The method used in these experiments of estimating the blood sugar was a modification of that of Lewis and Benedict,<sup>1</sup> and very similar to that of Epstein.<sup>2</sup>

### TECHNIC

Eight-tenths cubic centimeter of a 0.2 per cent. solution of sodium fluorid is placed in a small test tube. To this is added 0.2 c.c. of blood obtained with a capillary pipet from a finger prick; 1.5 c.c. of a saturated picric acid solution is then added drop by drop and well shaken.

If the picric acid is not added at once glycolysis takes place so the percentage is too low. We had to throw out one series of experiments before we discovered that this was the difficulty.

The mixture is then placed in a centrifuge tube, and centrifuged for two minutes. One cubic centimeter of the filtrate is then placed in a small Jena test tube to which is added 0.5 c.c. of a 10 per cent. sodium carbonate solution, a glass bead, and a drop of kerosene. This is slowly boiled over a microburner until but a few drops remain. Five to 10 drops of distilled water are added and the solution is heated for a moment; then it is poured into a Sahli hemoglobinometer tube and tested against a colorimeter tube of the same diameter containing a standard picramic acid solution so arranged that each line on the tube equals  $\frac{1}{4000}$  of 1 per cent. of sugar, or 1 mg. to 100 c.c. of blood.

McLeod and Pierce,<sup>3</sup> after working with the various methods, concluded that the technic of Lewis and Benedict was the best one they had used. The technic as we followed it is almost identical with the original, with the exception that we used but one-tenth as much blood and one-tenth of the various solutions used in the original. Hence our percentage of error is naturally larger, yet we found by paying careful attention to detail and by having all tubes cleaned with care, our percentage of error was no greater than that of usual clinical laboratory tests.

We frequently checked our instrument up with weighed glucose solutions. Glucose solutions, 0.05 and 1 per cent., were carefully prepared and were tested just as though they were blood.

Table 1 is one of a number of similar tables we made from time to time.

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\* Submitted for publication Oct. 25, 1916.

1. Lewis, R. C., and Benedict, S. R.: Jour. Biol. Chem., 1915, **20**, 61.

2. Epstein, A. A.: Jour. Am. Med. Assn., 1914, **63**, 1667.

3. Pierce, R. G.: Jour. Biol. Chem., 1915, **22**, 525.

TABLE 1.—SOLUTION CONTAINING 0.05 PER CENT. OR 50 MG. OF GLUCOSE TO 100 C.C.

Test No.	Mg. Sugar Per 100 C.c. Solution
1.....	48
2.....	53
3.....	49
4.....	49
5.....	50
6.....	53

TABLE 2.—SOLUTION CONTAINING 0.1 PER CENT. OR 100 MG. GLUCOSE PER 100 C.C.

Test No.	Mg. Sugar Per 100 C.c. Solution
1.....	95
2.....	100
3.....	100
4.....	105
5.....	98
6.....	102

## NORMAL BLOOD SUGAR

When reviewing the literature one is struck with the wide variations given by various authors as normal amounts of sugar in the blood (Table 3). None of these investigators examined more than twenty subjects, however.

TABLE 3.—AMOUNT OF BLOOD SUGAR FOUND BY VARIOUS AUTHORS

Author and Method	Blood Sugar, Per Cent.
Naunyn <sup>4</sup> with Abeles <sup>5</sup> .....	0.07 to 0.1
Klemperer <sup>6</sup> .....	0.06 to 0.11
Hollinger <sup>6</sup> with Knapp's.....	0.07 to 0.1
Bang <sup>7</sup> with Bang's Micro.....	0.1 to 0.11
Frank <sup>8</sup> with Bertrand's.....	0.06 to 0.11
Purjez <sup>9</sup> with Bertrand's.....	0.045 to 0.087
Kowarsky <sup>10</sup> with Kowarsky's.....	0.05 to 0.11
Strause <sup>11</sup> with Kowarsky's.....	0.04 to 0.088
Hopkins <sup>12</sup> with Bang's Micro.....	0.065 to 0.1

Table 4 is composed of results obtained by examination of the blood from one hundred convalescent male patients at the Los Angeles County Hospital who were chosen because of apparently normal digestive systems; and who were about to be discharged from the hospital. The specimens of blood were all taken before breakfast, or

4. Naunyn: Diabetes Mellitus, 1906.
5. Klemperer: Quoted by Bang, see Note 7.
6. Hollinger, A.: Deutsch. Arch. f. klin. Med., 1909, **92**, 217.
7. Bang, I.: Der Blutzucker, 1913, J. F. Bergman, Wiesbaden.
8. Frank, E.: Ztschr. f. physiol. Chem., 1910, **70**, 129.
9. Purjez, B.: Wien. klin. Wchnschr., 1913, **26**, 1420.
10. Kowarsky, A.: Deutsch. med. Wchnschr., 1913, **39**, 1635.
11. Strause, S.: Bull. Johns Hopkins Hosp., 1915, **26**, 292.
12. Hopkins, A. H.: Am. Jour. Med. Sc., 1915, **149**, 254.

at least three hours after meals, Hopkins having shown that the blood sugar had fallen to the same level three hours after meals as the level in a fasting or empty stomach.

TABLE 4.—RESULTS OF BLOOD SUGAR TESTS IN 100 MALE CONVALESCENTS

No.	Age	Per Cent. Sugar	Mg. Sugar in 100 C.c. Blood
1	49	0.044	44
2	20	0.044	44
3	36	0.049	49
4	59	0.049	49
5	28	0.049	49
6	48	0.053	53
7	28	0.053	53
8	23	0.055	55
9	23	0.055	55
10	55	0.055	55
11	..	0.055	55
12	44	0.056	56
13	39	0.057	57
14	49	0.057	57
15	31	0.057	57
16	24	0.057	57
17	35	0.057	57
18	23	0.058	58
19	43	0.058	58
20	45	0.058	58
21	49	0.058	58
22	69	0.058	58
23	65	0.060	60
24	61	0.060	60
25	..	0.060	60
26	65	0.061	61
27	66	0.061	61
28	66	0.061	61
29	52	0.061	61
30	50	0.061	61
31	42	0.061	61
32	35	0.061	61
33	44	0.061	61
34	51	0.061	61
35	40	0.063	63
36	60	0.063	63
37	59	0.064	64
38	29	0.064	64
39	52	0.064	64
40	35	0.064	64
41	20	0.066	66
42	40	0.066	66
43	62	0.066	66
44	34	0.066	66
45	15	0.066	66
46	55	0.067	67
47	58	0.068	68
48	65	0.068	68
49	33	0.068	68
50	30	0.070	70
51	24	0.071	71
52	..	0.071	71
53	25	0.071	71
54	43	0.071	71
55	28	0.071	71
56	43	0.071	71



TABLE 4—Continued

No.	Age	Per Cent. Sugar	Mg. Sugar in 100 C.c. Blood
57	66	0.071	71
58	20	0.071	71
59	27	0.071	71
60	..	0.073	73
61	40	0.073	73
62	30	0.073	73
63	21	0.073	73
64	41	0.073	73
65	60	0.073	73
66	35	0.074	74
67	13	0.074	74
68	22	0.074	74
69	26	0.075	75
70	60	0.075	75
71	..	0.077	77
72	45	0.077	77
73	36	0.077	77
74	26	0.077	77
75	25	0.077	77
76	55	0.077	77
77	18	0.078	78
78	..	0.078	78
79	..	0.079	79
80	12	0.079	79
81	..	0.079	79
82	41	0.080	80
83	71	0.081	81
84	73	0.082	82
85	24	0.082	82
86	42	0.082	82
87	18	0.082	82
88	42	0.082	82
89	65	0.084	84
90	34	0.087	87
91	62	0.088	88
92	36	0.090	90
93	..	0.090	90
94	65	0.090	90
95	52	0.092	92
96	52	0.100	100
97	36	0.100	100
98	49	0.110	110
99	41	0.115	115
100	42	0.120	120

In Table 4 it will be noted that the glucose varies from 44 to 120 mg. to 100 c.c. of blood. Outside of the normal variation of the amount of sugar in the blood, the two main factors producing the wide difference are errors in technic and disobedience of orders about food and drink. The maximum, minimum, and average compare very closely, however, with the figures of the authors quoted.

#### BLOOD SUGAR IN DIABETES

Table 5 gives data of eleven diabetics taken under the same conditions as the one hundred normals (Table 4).

TABLE 5.—BLOOD SUGAR IN ELEVEN DIABETICS

No.	Age	Per Cent. Sugar	Mg. Sugar in 100 C.c. Blood
1	..	0.044	44
2	..	0.060	60
3	..	0.082	82
4	62	0.082	82
5	..	0.104	104
6	60	0.110	110
7	46	0.123	123
8	60	0.126	126
9	62	0.132	132
10	62	0.143	143
11	..	0.143	143

The eleven subjects in Table 5 all had outspoken diabetes, yet the first six, on a single examination of the blood, would have to be classed within the group of normals. It is evident, then, that merely the estimation of the amount of glucose in the blood may give us no correct information of the ability of the patient to handle sugars.

#### TOLERANCE FOR SUGAR IN NORMALS

Baudouin<sup>13</sup> gave to six normal persons 100 gm. of glucose and examined the blood before, and also one hour and two hours after, taking the sugar. He found a distinct rise of blood sugar one hour after; but two hours after, it had dropped practically to normal.

Frank<sup>8</sup> found like results in eight subjects examined.

Tachau<sup>14</sup> found in nineteen subjects examined, no distinct rise one hour following 100 gm. of glucose.

Bang<sup>7</sup> found a distinct rise following 100 gm. of glucose, but no greater rise when 150 gm. were given.

Bing and Jacobson<sup>15</sup> found in neurasthenia a rise one hour after 100 gm. of glucose, dropping to normal, or nearly normal, at the end of the second hour.

In diabetic patients they found a greater rise than in normal subjects, but observed no definite relation between the blood sugar content and the glycosuria.

Bergmark<sup>16</sup> observed both adults and children, examining the blood before, one hour, two hours, and, in some, three hours after giving 10 to 100 gm. of glucose, lactose, saccharose and maltose.

The maximum rise after these various amounts was from one to one and one-half hours, with a fall to normal in two to three hours. It made little difference which variety of sugar was used.

13. Baudouin: Thèse de Paris, 1908.

14. Tachau: Deutsch. Arch. f. klin. Med., 1911, **102**, 297.

15. Bing, H. J., and Jacobsen, B.: Deutsch. Arch. f. klin. Med., 1914, **113**, 571.

16. Bergmark: Jahrb. f. Kinderh., 1914, **80**, 373.

TABLE 6.—METABOLISM OF SUGAR IN APPARENTLY NORMAL PERSONS

No.	Age	Fasting; Mg. Sugar per 100 C.c. of Blood	First Hour; Mg. Sugar per 100 C.c. of Blood	Second Hour; Mg. Sugar per 100 C.c. of Blood
1	40	37	38	62
2	49	44	73	77
3	..	46	63	56
4	..	49	63	63
5	28	49	101	68
6	..	50	114	97
7	28	52	71	68
8	48	52	71	68
9	..	55	92	71
10	..	55	89	95
11	23	55	93	88
12	55	55	123	96
13	..	57	85	71
14	24	57	89	115
15	35	57	84	55
16	..	58	73	67
17	44	58	121	88
18	65	58	82	66
19	..	60	74	66
20	..	60	114	119
21	50	61	93	70
22	52	61	127	121
23	66	61	129	111
24	..	62	93	88
25	40	62	118	107
26	..	63	83	68
27	59	63	114	94
28	35	64	119	99
29	..	66	93	77
30	..	66	121	121
31	58	68	137	121
32	43	71	71	55
33	28	71	83	71
34	24	71	104	99
35	..	71	204	123
36	25	71	102	93
37	27	71	102	88
38	60	72	119	110
39	41	72	117	148
40	20	72	123	115
41	56	74	108	99
42	18	77	79	72
43	45	77	125	110
44	56	77	88	80
45	26	77	104	71
46	25	77	127	115
47	55	77	91	73
48	..	77	91	91
49	..	79	70	79
50	42	82	110	66
51	18	82	110	66
52	..	84	115	111
53	34	87	123	110
54	..	87	78	85
55	..	89	110	81
56	..	93	107	99
57	..	90	88	77
58	..	110	159	110
Average		68	101	88



In order to ascertain the ability of a normal person to absorb and metabolize sugar, we gave 100 gm. of glucose or cane sugar to each of fifty-eight apparently normal persons. Table 6 gives their ages and the number of milligrams of sugar found in 100 c.c. of blood on a fasting stomach, and one hour and two hours after administering sugar.

In Table 6, except in Cases 1, 2, 14, 20, 39, 49 and 54, the sugar is higher at the end of the first hour than at the end of the second. The average is quite typical of the great majority. There is about a 50 per cent. rise during the first hour, with a drop of about one-half this during the second hour. These results correspond quite closely to the observations of Baudouin, Frank, and Bergmark.

#### TOLERANCE FOR SUGAR IN DIABETICS

The series of fourteen outspoken diabetics (Table 7) shows quite different results.

TABLE 7.—TOLERANCE FOR SUGAR SHOWN BY DIABETICS

No.	Age	Fasting; Mg. Sugar per 100 C.c. of Blood	First Hour; Mg. Sugar per 100 C.c. of Blood	Second Hour; Mg. Sugar per 100 C.c. of Blood
1	..	44	99	143
2	..	60	93	137
3	62	82	93	93
4	..	22	99	159
5	..	104	104	143
6	60	110	121	110
7	14	110	150	200
8	52	120	280	340
9	46	123	137	170
10	60	126	159	165
11	56	130	155	190
12	62	132	308	373
13	62	143	176	220
14	..	143	170	209

The technic of testing these diabetics (Table 7) was the same as that used in the normal subjects, yet the curve is quite different. Except in Cases 3 and 6, there is a greater amount of sugar at the end of the second hour than at the end of the first, and in most of the cases the rise is quite marked. In Cases 3 and 6 the patients had been under treatment for some time, and there had developed a distinct increase in their tolerance.

Two subjects were examined who we believe had some time previously had diabetes. The results are distinctly suggestive of a low tolerance.

#### REPORT OF CASES

Dr. E., aged 30, who four years previously had had a glycosuria, polyuria and a furunculosis which did not yield to any form of treatment until the

patient was placed on a low carbohydrate diet, had been free from glycosuria for many months. His blood during the fasting period contained 65 mg. per 100 c.c. One hour after 100 gm. of glucose was ingested there were 135 mg., and two hours after, it contained 90 mg. Here we have a normal amount of sugar during the fasting period, but after giving sugar, a marked rise, followed

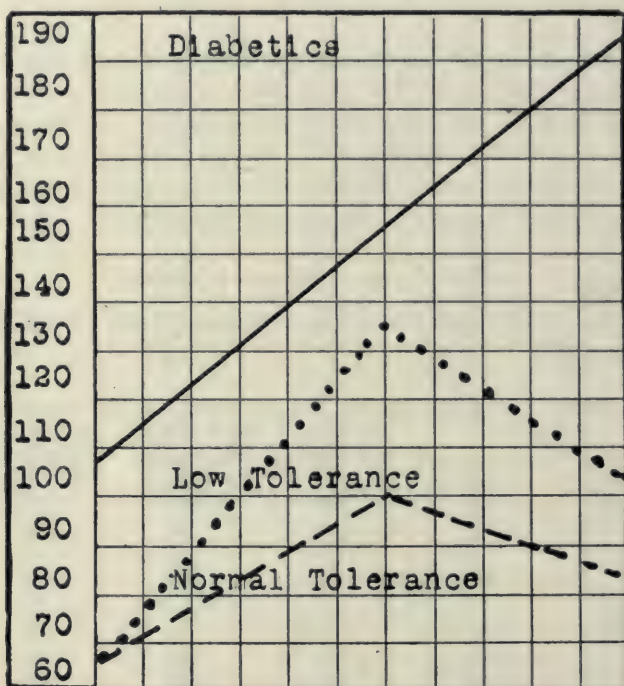


Chart showing variations from the normal in lowered sugar tolerance.

	Average	Fasting, Mg.	First Hour, Mg.	Second Hour, Mg.
Normal .....		68	101	88
Low tolerance.....		65	135	105
Diabetics .....		108	153	190

in two hours by a drop, which is still 50 per cent. above his fasting level. No glycosuria was present during this test.

Mrs. E., aged 34, who was still under treatment, but whose urine had been sugar-free for some time, had, during the fasting period, 65 mg. of sugar in 100 c.c. of blood; one hour after administering 100 gm. of glucose there were 132 mg., and two hours later the blood still contained 121 mg. This case shows distinctly a low tolerance for sugar. Glycosuria developed during this test.

Taking the average in these three groups of subjects, we constructed the accompanying chart, the curves of which show in a concrete manner the variations from the normal in those cases having a lowered tolerance for sugar.

## SUMMARY

1. We have made several hundred estimations of the amount of sugar in the blood by a simple technic which is a modification of Lewis and Benedict's method, and believe it to be reasonably accurate.

2. We measured the amount of sugar in the blood of 100 apparently normal subjects, finding the minimum to be 44 mg., the maximum 120 mg., with the average 70 mg. sugar per 100 c.c. of blood.

3. We estimated the tolerance of fifty-eight normal subjects for sugar, finding the maximum amount of sugar in the blood to be at the end of the first hour following the ingestion of 100 gm. of glucose, with a drop almost to normal at the end of the second hour.

4. We estimated the tolerance for sugar in fourteen cases of outspoken diabetes, finding more sugar in the blood at the end of two hours after the ingestion of 100 gm. of glucose than at the end of one hour, as in the normals.

5. We estimated the tolerance for sugar in two subjects who had apparently had diabetes, finding a marked rise during the first hour, with but a moderate fall during the second hour.

## CONCLUSIONS

1. From our observations we conclude that in a real or moderately severe case of diabetes the blood sugar is higher two hours after giving 100 gm. of glucose than it is one hour after.

2. In the milder forms of diabetes the blood sugar will be found normal, but following the administration of 100 gm. of glucose the rise in blood sugar will be greater than normal, and especially will this rise be sustained well into the end of the second hour.

3. In subjects having a low tolerance for sugars, the rise following the ingestion of 100 gm. of glucose will not be so high as in diabetes, yet it is distinctly higher than normal, and the height is well sustained into the end of the second hour.

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# THE HUMAN AND ANIMAL LIVER AFTER ALCOHOL\*

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## INTRODUCTION

Long before the microscope came to play the important rôle in pathology that it now does, the liver, in common with other large organs, presented changes for gross study. Thus Laennec<sup>1</sup> in his work on auscultation, published in 1829, mentions in a case of ascites the presence of a small liver with coarsely granular surface. Owing to the yellowish color of the organ he applied the term *cirrhose*.

The term cirrhosis has survived, and, like other terms that are not applied to given conditions of definite etiology, it has not always been used in the same way. We do not hesitate to call the condition in which there is a complete loss of liver lobules with the formation of wide bands of connective tissue, a cirrhosis, but if there is a moderate increase of connective tissue about the portal areas and along the interlobular lines, the term sclerosis is likely to be preferred. Many authors, however, speak of the moderate increase of connective tissue that may follow, for instance, a tuberculous process in the liver, as a cirrhosis. At present, perhaps, the commonest usage is to apply cirrhosis to the more extensive grades of sclerotic change in the liver.

Following the observation of Laennec, a large mass of literature accumulated dealing with the etiologic factors that produce or appear to produce liver cirrheses. For the most part it is difficult to place the credit for our present knowledge of the causative agents. MacCallum<sup>2</sup> and Kretz<sup>3</sup> have pointed out the way in which regeneration of the cells gives rise to large islands of liver cells with complete loss of lobulation; Levaditi and others are responsible for our ability to demonstrate the relationship between the *Treponema pallidum* in the liver and the sclerotic changes present; while Mallory<sup>4</sup> has shown that a characteristic hyaline (Fig. 1) change is present in the cytoplasm of the liver cells of cirrhosis of a certain type.

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2. MacCallum, W. G.: Regenerative Changes in Cirrhosis of the Liver,

1. Laennec, R. T. H.: *De L' Auscultation Mediate*, 1819, **1**, 368.  
Jour. Am. Med. Assn., 1904, **43**, 649.

3. Kretz, R.: *Ueber Lebercirrhose*, Wien. klin. Wchnschr., 1900, **12**, 271.

4. Mallory, F. B.: *Bull. Johns Hopkins Hosp.*, 1911, **22**, 69.

The etiology of those cirrhoses, in which the causative agent is present in the tissue as a morphologic element, was the first to be established, and there is at present, therefore, much greater certainty in regard to the mechanical (carbon and hemosiderin deposits), infectious (colon and possibly other ascending gall-duct infections) and syphilitic varieties than there is concerning the toxic. Leaving out of account the central scleroses produced by certain bacterial toxins and which may be induced by chloroform, the etiology of toxic liver lesions is yet obscure. This applies to the sclerosis following acute yellow atrophy.

Owing to the frequent history of alcoholism, ethyl alcohol was looked on as an active factor in the production of liver cirrhosis shortly after the observations of Laennec. Later, cirrhotic livers were found in infants and youths that had never used alcohol. This confused matters until the mechanical, syphilitic, and infectious varieties were clearly defined. Now, as during the earlier observations, a feature of these cases of unknown etiology is the excessive use of alcohol, especially of distilled liquors.

#### HUMAN CIRRHOSIS OF UNKNOWN ETIOLOGY

In a series of thirty recent cases of cirrhosis of unknown etiology coming to necropsy from the wards of the Boston City Hospital, the clinical records of twenty-eight make note of alcoholism. On the other hand, the majority of those dying from chronic alcoholism in the alcoholic wards of the hospitals show no abnormal changes in the liver. The excessive use of alcohol does not, therefore, always produce liver lesions in the human, while some workers along this line are willing to say that it never does. Many say that it does so indirectly by causing lesions in the gastro-intestinal tract, with a resulting autointoxication, while others say that alcoholic cirrhosis results from some impurity in the liquors. The most that can be said from the clinical study of human cases is that the majority of those with a cirrhosis not infectious or syphilitic (and not mechanical) use or have used alcohol to excess.

These cases were chosen from necropsies performed at the Boston City Hospital by examining the sections of liver for hyalin-containing cells. This examination excluded cases of sclerosis resulting from an attack of so-called acute yellow atrophy, in which the hyaline degeneration is not present and the lesion is not active and progressive. The microscopic examination makes possible the elimination of mechanical lesions from carbon or other pigment deposits and lesions from the extension of an infectious process up the bile passages, while the microscopic and gross appearance, together with the clinical history and clinical laboratory tests enable one to be quite sure that no cases of syphilis are included. These cases, then, are instances of cirrhosis

of unknown etiology, most of which are more or less alcoholic. The chief interest in the series is that the similarity in the microscopic appearance points to one toxic agent or to toxic substances acting in practically the same way. Thirty cases were taken; of these, Cases 1 to 5 show very little sclerosis, 6 to 9 are in the transition stage, while 10 to 30 are late, with extensive sclerosis. Cases 1 to 10 show each step in the process that ends with the typical "hobnail" liver.

The hyaline network in the affected cells has been accurately described by Mallory. This change is fully as evident as the nuclei of the cells themselves (Fig. 1). It is not present in cirrhosis of known etiology and is lacking in the degenerating cells of other liver affections. An interesting feature of the histologic study of the early cases (1 to 9) is that although the lobules are irregularly involved, the process is more severe toward the central hepatic vein.

To summarize, the thirty cases of this series are not cirrhoses of mechanical, infectious or syphilitic origin in the sense that these terms are used in the foregoing. Although the liver lesion is of toxic origin, it differs from acute yellow atrophy where the lesion is not progressive and where there is no hyaline degeneration of the liver cells, and from the central necrosis and sclerosis that may follow chloroform or bacterial toxins, where the lesion is not progressive and there are no hyaline-containing cells.

#### REPORT OF CASES

CASE 1.—M. F., woman, aged 28. The patient's sister says "patient has been drinking a good deal of late;" clinical diagnosis, alcoholic neuritis; patient died eleven days after admission to the hospital.

Liver weighs 1,800 gm.; the surface is smooth; microscopically the normal lobulation is present; there is no necrosis and no sclerosis. A few lobules have a single cell with a typical hyaline network. The other cells are normal except for a slight edema of those near the central vein. In all of the following cases there has been a loss and a failure of regeneration of a sufficient number of cells to lead to areas of sclerosis in the lobules or to sclerosis of entire lobules.

CASE 2.—J. W., man, aged 33. During the last few weeks the patient has grown weaker and at his lodging house he was often helped to his room when intoxicated. He was admitted to the hospital in an unconscious condition and died on the day of admission.

Weight of the liver 1,730 gm.; surface is smooth; microscopically, the lobulation is normal. There is a slight sclerosis which differs from the usual central toxic sclerosis (chloroform, streptococcus toxin, etc.) in that it is not confined to the zone about the central hepatic vein, but extends irregularly at one or more points toward the portal tissue and may even reach the periphery of the lobule.

On examining these areas of sclerosis with an oil immersion lens, a majority of the cells are found to have disappeared, not only from the inner ends of groups of trabeculae, but also from the intermediate and outer portions. Some of the liver cells remaining in these trabeculae are normal while others contain hyalin.

CASE 3.—F. W., man, aged 41. The patient came to the hospital with a history of recent hard drinking, developed delirium tremens and died on the seventeenth day after admission.



Weight of the liver 1,975 gm.; surface is smooth. Microscopically the liver is like that in Case 2.

CASE 4.—W. G., man, aged 49, had been very alcoholic, and for two weeks had had facial erysipelas; entered the hospital delirious and died on the second day following admission.

Weight of the liver 2,680 gm.; the surface is granular. Microscopically the liver is like the organs in Cases 2 and 3, except that the loss of cells from trabeculae is very diffuse and the number of hyalin-containing cells in every part of the lobule is practically the same.

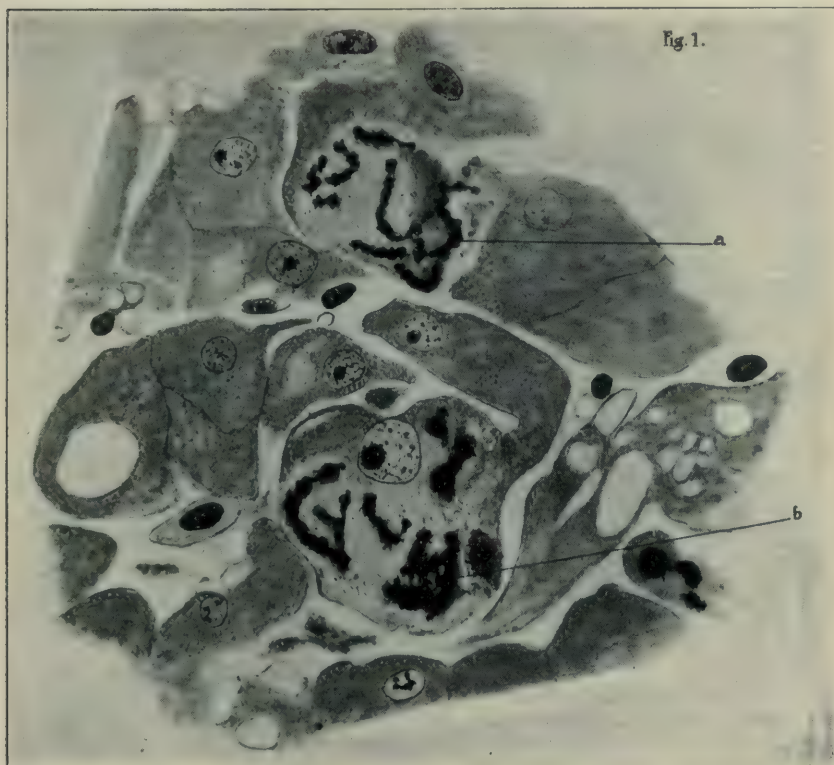


Fig. 1.—High power magnification of the liver from a case of human cirrhosis of unknown etiology (Case 7). *a* and *b* are two cells showing the hyaline degeneration of the cells that characterizes this variety of cirrhosis. *b* shows nucleus intact which is the case in a considerable number of the cells so affected. Such cells are very often invaded by leukocytes, but neither of these cells has been so involved.

CASE 5.—G. T., man, aged 35, drank three to four glasses of whisky daily; died on the fifth day after admission.

Weight of the liver 2,560 gm.; surface of the liver is smooth. Microscopically the liver is like the organs in Cases 2 and 3.

CASE 6.—M. B., woman, aged 48, drank one pint of whisky per week; also beer. She was intoxicated when admitted to the hospital and died one month from date of admission.

Weight of the liver 2,400 gm.; surface smooth. Here the process is somewhat more extensive. The loss of cells from trabeculae reaches from central veins to portal areas and in many lobules the majority of the cells are gone. Most of the section is made up of sclerosed lobules with small islands of cells about the portal areas. In places an island of normal liver cells lies only on one side of a portal area.

CASE 7.—A. W., woman, aged 35, used some alcohol; in bed for one week with vomiting and diarrhea; no jaundice; died six days after admission to the hospital.

Weight of the liver 1,670 gm.; surface is granular. Microscopically the amount of sclerosis is the same as in Case 6.

CASE 8.—M. T., woman, aged 55, drank one pint of gin daily; had been a heavy drinker for three years; had jaundice for four weeks; died eighteen days after admission.



Fig. 2.—High power magnification of the liver from a rabbit that received 4 gm. lead acetate forty-eight hours before being killed. *a* and *b* are two cells that show a fine granulation in the form of a network. Many of these cells become invaded by leukocytes and undergo necrosis. This network gives a black precipitate when treated with an alkaline sulphid, a reaction that differentiates this change from that shown in Figure 1.

Weight of the liver 1,800 gm.; surface is granular. Microscopically this liver is typically in the stage of transition from a sclerosis of lobules to a sclerosis with a loss of lobules and the formation of islands of liver cells. The tendency for these islands of unaffected cells to lie about the portal tissue is shown by the two preceding cases. In some lobules some irregular sclerosis about the central hepatic vein extends to the portal tissue, and in this way circular bands made up of one to several portal tracts are formed. These bands inclose islands of liver cells made up of the parts of one or more lobules next the portal tissue.

CASE 9.—L. B., man, aged 58, had never been sick; never used liquor to excess, but drank more during the previous month; jaundiced for one month; gradually weakened and died eighteen days after admission to the hospital.

Weight of the liver 2,750 gm.; surface is granular. Microscopically, sections show beautifully the inclosing of peripheral portions of lobules by bands made up of portal areas connected with the irregular central scleroses.

CASE 10.—B. T., man, aged 50, drank three to four whiskies and four to five beers per day; eight months previous to admission he felt tired in the morning and would take a glass of brandy to stop his vomiting; for two months the abdomen had been enlarging and he had had diarrhea; he was slightly jaundiced on admission and died eight days later.

Weight of the liver 1,220 gm.; surface is granular. Microscopically this liver is made up of islands of liver cells of very irregular shape and size, surrounded by and cut into by rather narrow connective tissue bands. A few of the islands have portal areas at or near their centers.



Fig. 3.—High power magnification of the liver from a guinea-pig (No. 46) that had received maximum doses of ethyl alcohol. *a* is a necrotic liver cell lying between two sinusoids. A few such cells represent the greatest damage that can be effected with alcohol. A few cells lost in this way are replaced perfectly.

Reference to the preceding cases offers a clear explanation of the method of formation of these islands. This case differs from the preceding ones in being a more advanced stage of the sclerotic process. That is, the areas of sclerosis involve all lobules to such an extent that all the central hepatic veins and most of the portal areas are included in the fibrous bands.

The islands of normal liver cells are originally made up of a segment of a lobule or the parts of several lobules that abut on portal tissue. That regeneration has taken place in the original islands is



shown by the presence of islands larger than any normal lobules. Mitoses of liver cells may be found in many of these cases, but frequently it is difficult microscopically to find evidence of regeneration.

These islands are subject to hyaline degeneration just as the lobules are in the earlier stages of the process. For this reason, and also owing to the inclusion of small focal necroses in persisting parts of lobules, the islands of liver cells are very diffusely cut into by connective tissue bands that are narrower than those surrounding the



Fig. 4.—Low power magnification of the liver from a rabbit that had never been made the subject of any experiment. This process occurs naturally in rabbits and is found in a large percentage of old animals. The necrosis at *a* and the leukocytic infiltration at *b* are followed by a fibrosis such as was met with in Animal 221. This is the change that has been described by those who claim to have produced a cirrhosis in animals by means of ethyl alcohol.

islands. The narrowness of the bands and this irregular cutting up of the islands is often sufficient to differentiate this variety of cirrhosis from the cirrhosis that follows what is known as acute yellow atrophy.

CASE 11.—A. D., woman, aged 46, drank one bottle of beer daily; died thirty days after admission.

Weight of the liver 1,870 gm.; surface is granular. Microscopically it is like the liver in Case 10.

CASE 12.—I. C., woman, aged 50. No history; weight of the liver 1,260 gm.; surface is granular. Microscopically it is like that of Case 10 except that many islands are larger.

CASE 13.—R. M., man, aged 47, drank one pint of whisky and three to four beers daily; for three weeks the abdomen had been increasing in size; some jaundice; died ten days after admission.

Weight of the liver, 1,720 gm.; surface is granular. Microscopically it is like that of Case 10.

CASE 14.—C. D., man, aged 46, drank three to four glasses of whisky per day; for three weeks had been growing weak; admitted to the hospital and died fifteen days later.

Weight of the liver 2,350 gm.; surface is granular. Microscopically the connective tissue bands are narrow and ill-defined, and included in these bands there are a great many cells containing hyalin.

CASE 15.—J. N., man, aged 34, drank much whisky during the previous ten years; had stomach trouble and "dry heaves" for years; delirious for three days before entrance to the hospital, and died ten days later.

Weight of the liver 3,029 gm.; surface is granular. Microscopically it is like that in Case 10, except that the islands are larger.

CASE 16.—A. M., man, aged 50, drank seven to eight beers or whiskies daily; quit work three months previous to admission on account of shortness of breath; died twenty-one days after admission to the hospital.

Weight of the liver 1,750 gm.; surface is granular. Microscopically it is like that of Case 10.

CASE 17.—N. H., woman, aged 38, used alcohol for twenty years; in excess for one year; swelling of the abdomen for five weeks; jaundice present; died twelve days after admission.

Weight of the liver 3,180 gm.; surface is smooth. Microscopically it is like the liver in Case 10, except that the connective tissue bands are very narrow and the islands very large. The liver cells contain a great amount of fat.

CASE 18.—R. M., woman, aged 32, drank a half pint of gin per week; for six weeks especially sick; when admitted had an alcoholic breath; died thirteen days after admission.

Weight of the liver 1,310 gm.; surface is granular. Microscopically it is like that of Case 10.

CASE 19.—E. S., man, aged 37, drank twelve to twenty-five glasses of beer daily and brandy occasionally; for the previous five months he complained of weakness; the previous ten days he was jaundiced and had pain in the epigastrium; died two days after admission.

Weight of the liver 2,900 gm.; surface is granular. Microscopically like the liver in Case 10.

CASE 20.—N. H., woman, aged 38. For the previous nine years the patient used much alcohol; there was jaundice and pain in the back for six months; died one day after admission.

Weight of the liver 2,990 gm.; surface is granular. Microscopically like that of Case 10.

CASE 21.—M. O., woman, aged 60. Admitted unconscious following a fall; no history.

Weight of the liver 1,270 gm.; surface is granular. Microscopically like the liver in Case 10.

CASE 22.—W. B., man, aged 50, drank beer, alcohol, and whisky for years; jaundiced for three months; increasing ascites and edema of the legs.



Weight of the liver 1,555 gm.; surface of the liver is granular. Microscopically like that of Case 10.

CASE 23.—M. F., man, aged 51; alcohol used; admitted in a semiconscious condition with an alcoholic breath; died four days later.

Weight of the liver 1,440 gm.; surface is granular. Microscopically like that of Case 10.

CASE 24.—F. M., man, aged 56, had a "spree" once in two months; vomiting; jaundice; died three days after admission.

Weight of the liver 2,100 gm.; surface is granular. Microscopically like the liver in Case 10 except that the sclerosis is somewhat less advanced (a few lobules may be found).

CASE 25.—G. M., man, aged 29, had used alcohol steadily and to excess for three to four years; well until three months prior to admission when vomiting and swelling of the abdomen began; died thirty-four days after admission.

Weight of the liver 2,425 gm.; surface is granular. Microscopically like that of Case 10.

CASE 26.—E. S., man, came into the hospital complaining of acute rheumatism in the right knee; developed delirium tremens and died twelve days after admission; the clinical diagnosis was alcoholism.

Weight of the liver 2,200 gm.; surface is smooth. Microscopically like the liver in Case 10.

CASE 27.—N. H., woman, aged 41, drank wine every morning; died six days after admission.

Weight of the liver 2,650 gm.; surface is granular. Microscopically like that in Case 10.

CASE 28.—C. L., woman, aged 49. Diarrhea, loss of weight, vomiting and enlargement of the abdomen for one month before entrance. Husband said the patient used alcohol.

Weight of the liver 3,080 gm.; surface is granular. Microscopically like that of Case 10.

CASE 29.—F. P., man, aged 33. Run down for the previous three months; drinking heavily; alcohol in excess for years; since entrance to hospital, delirious.

Weight of the liver 5,335 gm.; surface is granular. Microscopically like the liver in Case 10, except for cells containing a large amount of fat.

CASE 30.—C. T., woman, aged 53. In bed for two months with vomiting; not asked about alcohol; died thirty-five days after admission to the hospital. After the necropsy the husband stated that the patient drank much brandy.

Weight of the liver 1,380 gm.; surface is granular. Microscopically like that of Case 10.

Anatomically, Cases 1 to 9, inclusive, are not ones of frank cirrhosis. They are included in the series because the cells show a characteristic hyaline degeneration. This hyaline change is present in this variety of cirrhosis of unknown etiology, and is found in no other condition, except, rarely, in an alcoholic (Cases 1 to 9) in which the changes point to the active degenerative stage that ends in a cirrhosis. Whatever the toxic substance active in this cirrhosis, the hyaline change appears to be the degenerative lesion.

Accepting these cases as different stages of the process that ends in this variety of cirrhosis, especial interest attaches to the microscopic



changes. In all the earlier cases it is seen that, although the degenerative change strikes quite irregularly throughout the lobule, it makes its first appearance toward the center, and this is the part first lost in the process that extends irregularly and finally destroys the entire normal lobulation.

The descriptions that are available for study deal at length with a fully developed cirrhotic liver, while the degenerative changes that produce this anatomic rearrangement of the liver structure are guessed at by inference. Degeneration and regeneration in the cirrhotic liver are described at length, but little is said in regard to the active lesion that is so severe that the entire normal lobulation is lost. As in other processes that end in sclerosis, the early active stage, summarized in the preceding paragraph and detailed in Cases 1 to 9, inclusive, is the important one to study from the etiologic, anatomic and therapeutic point of view. If the causative factor can be definitely established, it is possible that during this stage the progress of the lesion may be stopped.

The common observation that the livers of those dying from acute and chronic alcoholism show, in the great majority of cases, no change except an abundance of fat in the liver cells, is found true in the study of the necropsies performed at the Boston City Hospital on those dying from alcoholism during fifteen years previous to 1915. There is no question that alcohol as used by chronic alcoholics produces no liver lesion in the great majority of cases.

Another point that indicates that the use of alcohol acts indirectly or not at all, is the length of time intervening between admission of the patients to the hospital and their death (more than one month in four of the thirty cases). Since an active and extensive degenerative change is present in the liver of a person one month after his last dose of alcohol, the degenerative change either persists for this length of time or the alcohol acts indirectly or not at all on the liver cells. The rapidity of necrosis and solution, and the invasion of affected cells by leukocytes, ending in their rapid removal, makes it very unlikely that seriously injured cells persist in that state for so long a time.

#### THE ANIMAL LIVER AFTER DOSAGE WITH ALCOHOL

With a substance so simple chemically as alcohol, one would expect the action on the lower animals to be similar to that in the human, and numerous attempts have been made to clear up the question of so-called alcoholic cirrhosis by administering alcohol to animals. Out of one list of thirty workers who gave ethyl alcohol to animals, seventeen produced no lesion in the liver, while thirteen produced a cirrhosis. Those that produced a cirrhosis worked with rabbits. Rabbits frequently show an increase in round cells in the periportal tissue and

along the interlobular lines (Fig. 4). In this way islands of liver cells and liver lobules become surrounded by fibroblastic tissue. There seems to be some contention as to whether this process, occurring spontaneously in rabbits, should be called a cirrhosis, but disregarding this technicality, it does correspond to the reproductions and descriptions representing the cirrhosis said to be produced by alcohol in rabbits.

A search through the reports of these investigators is a rather barren one. Those reporting positive results have, as mentioned above, made use of rabbits, and their positive results are based on descriptions that correspond to the natural lesion found in these animals. Although investigators now agree that an extensive necrosis and loss of liver cells leads to the cirrhotic liver as an end result, the majority of the experimental workers have made little or no attempt to demonstrate this active process that leads to the sclerosis. In such a case as this in which all the factors used in the experiments are so accessible, it seems largely a waste of time to go into an extensive critical analysis of the literature.

Since alcohol appeared to be the most obvious factor, it was given persistently to animals until it was found that no lesion, however slight, could be produced. Later, other substances were tried. A total of 300 animals were used in the series of experiments. Of these, 125 were given ethyl alcohol (Table 1).

*Alcohol.*—The alcohol was given by the stomach, subcutaneously, intraperitoneally, by the rectum, by injection into the common bile duct, and by injection directly into the liver. In the animals receiving the alcohol per os, 80 per cent. alcohol was usually given daily or on alternate days and in doses sufficiently large to render the animal unable to stand. Usually it was given on an empty stomach.

In the liver of no animal is there a necrosis and degeneration of liver cells such as is seen in the human cases. Direct injection into the liver and injection into the common duct gave a focal necrosis of course, but the necrotic cells show no peculiarity. In the other animals a few necrotic cells (Fig. 3), usually less than twenty-five per microscopic lobule, are present, but such small numbers of scattered necrotic cells are found in the livers of animals dying from a variety of causes. Animals that died were subjected to necropsy and the organs fixed within one hour, and usually within ten minutes, after death. This is an absolutely necessary precaution, especially in small animals.

The length of time during which the alcohol was given was not great. If it is not possible to produce any degenerative change by the most intensive dosage during as long a period as a month, there is little possibility that alcohol ever produces a lesion in animals that leads to cirrhosis.

Of the remaining 175 animals that received substances other than alcohol, none showed liver lesions similar to those present in human alcohol cirrhosis, except the ones that were given large doses of lead.

*Lead.*—The liver lesion (Fig. 2) that is produced by lethal or slightly sublethal doses of lead has been described in detail by me.<sup>5</sup> The hyaline net-

5. McJunkin, F. A.: The Local Action of Lead, Jour. Med. Research, 1915, 32, 271.

TABLE GIVING DATA OF AUTHOR'S ANIMAL EXPERIMENTS

No.	Animal	Number of Doses	Alcohol, per Cent.	Amount, C.c.	Period Given, Days	Liver <sup>1</sup>
41-45	Guinea-pig.....	39 to 78	30	5	42 to 86	Negative
46	Guinea-pig.....	12	60	4	24	Occasional necrotic cell
56	Guinea-pig.....	15	60	4	30	Negative
58	Guinea-pig.....	24	"	8	45	Negative
59	Guinea-pig.....	20	"	8	43	Negative
62	Guinea-pig.....	8	80	5	17	Negative
63	Guinea-pig.....	8	80	5	23	Negative
117	Guinea-pig.....	29	80	5	62	Negative
118	Guinea-pig.....	34	80	5	88	Negative
119	Guinea-pig.....	21	80	5	45	Negative
120	Rabbit.....	..	95 <sup>3</sup>	0.5	..	Focal area of necrosis
124	Guinea-pig.....	23	80	5	50	Negative
125	Guinea-pig.....	23	80	5	50	Negative
126	Guinea-pig.....	23	80	5	50	Negative
127	Guinea-pig.....	68	80	5	44	Occasional necrotic cell
128-138	Guinea-pig.....	22 to 80	80	5	24 to 42	Negative
140-142	Guinea-pig.....	18 to 33	80	5	18 to 33	Negative
146	Cat.....	21	80	12	21	Negative
148	Cat.....	24	80	12	52	Negative
149	Cat.....	15	80	12	34	Few necrotic cells
150	Cat.....	11	80	12	23	Negative
152	Cat.....	6	80	12	15	Negative
153	Cat.....	7	80	12	22	Negative
154-158	Cat.....	5 to 12	95	8	10 to 62	Negative
169-175	Rabbit.....	3 to 10	80	6	6 to 11	Negative
187	Cat.....	..	80 <sup>4</sup>	1	..	Focal necrosis
199	Guinea-pig.....	..	40 <sup>5</sup>	5	..	Negative
200	Guinea-pig.....	..	20 <sup>6</sup>	1	..	Negative
201	Guinea-pig.....	2	40 <sup>7</sup>	..	2	Negative
202	Guinea-pig.....	2	40 <sup>7</sup>	..	2	Negative
206	Guinea-pig.....	3	40	5	3	Negative
208	Guinea-pig.....	31	40	5	38	Occasional necrotic cell
210	Guinea-pig.....	1	40	5	1	Negative

1. The condition of liver is not recorded in a few cases not fixed within ten minutes after death. Immediate fixation is of the utmost importance.

2. Port wine.

3. Directly into liver. The alcohol was always given per os on a fasting stomach except when otherwise specified.

4. Injected into hepatic duct; animal killed in three days.

5. Injected into mesenteric vein; animal died immediately.

6. Injected into mesenteric vein; animal died in forty-eight hours.

7. Injected into rectum.



TABLE GIVING DATA OF AUTHOR'S ANIMAL EXPERIMENTS—(Continued)

No.	Animal	Number of Doses	Alcohol, per Cent.	Amount, C.c.	Period Given, Days	Liver <sup>1</sup>
211	Guinea-pig.....	1	40	5	2	Negative
214	Rabbit.....	10	40 <sup>8</sup>	7	10	Negative
215	Guinea-pig.....	2	40 <sup>8</sup>	6	3	Negative
216	Guinea-pig.....	2	40 <sup>8</sup>	4	2	Negative
217	Rabbit.....	1	40	15	2	Negative
218	Dog.....	29	40	60	61	Negative
219	Rabbit.....	3	40	15	4	Negative
220	Guinea-pig.....	8	40	3	9	Negative
221	Rabbit.....	2	40	20	2	Surface of liver rough; microscopically marked sclerosis; negative
222	Rabbit.....	3	40	20	4	Negative
223-227	Guinea-pig.....	10 to 64	20 <sup>8</sup>	4	23 to 72	Negative
228-231	Rat.....	3	20 <sup>8</sup>	8	3	Negative
243	Guinea-pig <sup>9</sup> .....	..	..	..	..	Necrotic foci involving several lobules
244	Guinea-pig <sup>9</sup> .....	..	..	..	..	Necrotic foci involving several lobules
246	Guinea-pig <sup>9</sup> .....	..	..	..	..	Necrotic foci involving several lobules
247	Guinea-pig <sup>9</sup> .....	..	..	..	..	Necrotic foci involving several lobules
250	Guinea-pig <sup>9</sup> .....	..	..	..	..	Necrotic foci involving several lobules
252-263	Guinea-pig <sup>9</sup> .....	..	..	..	..	Necrotic foci involving several lobules
270	Rabbit.....	8	80 <sup>10</sup>	14	14	Negative
272-278	Guinea-pig.....	8	80 <sup>10</sup>	14	1 to 16	Negative
279	Guinea-pig.....	3	80 <sup>11</sup>	4	6	
280	Guinea-pig.....	2	80 <sup>11</sup>	4	6	Central necrosis
281	Guinea-pig.....	2	80 <sup>11</sup>	4	6	
282	Guinea-pig.....	2	80 <sup>11</sup>	4	6	Central necrosis
283	Guinea-pig.....	9	80 <sup>11</sup>	4	30	Slight central necrosis
284	Guinea-pig.....	18	80 <sup>11</sup>	4	54	Negative
285	Guinea-pig.....	27	80 <sup>11</sup>	4	60	No necrosis
286	Guinea-pig.....	33	80 <sup>11</sup>	4	60	Negative
287	Guinea-pig.....	40	80 <sup>11</sup>	4	80	Negative
288	Guinea-pig.....	36	80 <sup>11</sup>	4	80	Marked central necrosis
289	Guinea-pig.....	23	80 <sup>11</sup>	4	92	Negative
290	Guinea-pig.....	23	80 <sup>11</sup>	4	92	
291	Guinea-pig.....	24	80 <sup>11</sup>	4	59	Negative

1. The condition of liver is not recorded in a few cases not fixed within ten minutes after death. Immediate fixation is of the utmost importance.

8. Injected subcutaneously.

9. Injected 0.25 c.c. 80 per cent. alcohol into common duct after ligation of cystic duct.

10. After chloroform anesthesia lasting two hours.

11. Alcohol containing 1 per cent. chloroform.

TABLE GIVING DATA OF AUTHOR'S ANIMAL EXPERIMENTS—(Continued)

No.	Animal	Number of Doses	Alcohol, per Cent.	Amount, C.c.	Period Given, Days	Liver <sup>1</sup>
292	Guinea-pig.....	34	80 <sup>12</sup>	4	69	Central necrosis
293	Guinea-pig.....	34	80 <sup>12</sup>	4	69	Central necrosis
294	Guinea-pig.....	34	80 <sup>12</sup>	4	69	Slight central necrosis
295	Guinea-pig.....	35	80 <sup>12</sup>	4	80	
296	Guinea-pig.....	35	80 <sup>12</sup>	4	80	
297	Dog.....	24	80 <sup>13</sup>	7	77	Marked central necrosis
298	Dog.....	25	80 <sup>13</sup>	7	76	Central necrosis
299	Dog.....	14	80 <sup>13</sup>	7	65	Central necrosis
300	Dog.....	60	80 <sup>13</sup>	7	60	Slight central necrosis

12. With 3 per cent. chloroform.

13. With 4 per cent. chloroform.

work present in the liver cells of animals receiving lead salts blackens when treated with an alkaline sulphid. The hyalin-containing cells from cases of human cirrhosis do not give this reaction. Altogether, seventy-six animals were given lead salts and their livers examined microscopically.

*Chloroform*.—Fifteen animals were given chloroform in doses of sufficient size to produce slight to extensive central necrosis. These animals were used to control animals that received both alcohol and chloroform.

*Pancreatin*.—Twenty-nine animals received varying strengths and forms of pancreatin per os and by injection into the bile passages. The liver was negative so far as changes suggesting those present in human cirrhosis are concerned.

*Bacterial Toxins*.—Sixteen animals were given by the mouth the cultural products of a number of intestinal bacteria alone and in combination with alcohol. Liver negative.

*Acetaldehyd*.—Eight animals. Liver negative so far as changes like those present in human cirrhosis are concerned.

*Formaldehyd*.—Seven animals; liver negative.

*Other Substances*.—Pepsin, hydrochloric acid, chloral hydrate, acetone, acetic acid, sodium acetate, stannous chlorid, copper sulphate, mercuric chlorid, bismuth and Rochelle salts. Twenty-four animals; liver negative.

#### SUMMARY AND CONCLUSIONS

After the elimination of cases of cirrhosis of known etiology of the mechanical, infectious and syphilitic varieties, many cases fall into a class of unknown etiology. These cases appear to be of toxic origin, but differ from the central scleroses produced by known bacterial toxins and by chloroform, as well as from the cirrhosis that may follow acute yellow atrophy. Alcohol is suggested as the causative agent by the history of these cases, but there is no further evidence to indicate that it injures the liver. On the other hand, the frequently-mentioned fact that the percentage of cirrhosis among those dying

from chronic alcoholism is only slightly greater than it is among those dying from all other causes, indicates that this substance has no direct action on the liver.

A point brought out in the histologic study of a series of human cases is that there is a very active and characteristic degeneration and necrosis of liver cells present even as long as a month after the patient received his last alcohol.

A further reason for thinking that alcohol does not act directly on the liver to produce a cirrhosis is that it does not injure the liver in the lower animals. It not only does not produce a cirrhosis, but it produces no noteworthy lesion whatsoever of the liver cells.

If alcohol works no damage on the liver, or does so only indirectly, the question of the number of factors at work in the production of these cases of cirrhosis of unknown etiology arises along with the problem of the nature of the hepatic poison. In regard to the first of these questions, the uniformity of the cellular changes, especially the characteristic hyaline degeneration of the hepatic cells, and the very irregular cutting up of the lobules and islands by rather narrow sclerotic bands, point to a single causative agent or to poisons acting alike.

As to the nature of the poison, there is little evidence now at hand. Of a number of substances given to animals, lead is the only one that produces a hyaline change. The failure of the hyalin of human liver to react with an alkaline sulphid, as does that produced by lead in experimental animals, indicates that lead is not the active agent in this variety of cirrhosis. The uniformity of the lesion of these cases brought out in this study at least tends to show that there is here a variety of cirrhosis, the etiology of which is for future solution. The so-called alcoholic cirrhosis is not produced directly by ethyl alcohol, that is, the liver is not injured by the alcohol carried to it through the blood or lymph.

Fourth Street and Reservoir Avenue.



# THE INFLUENCE OF THE RADIATIONS FROM KROMAYER'S MERCURY QUARTZ LAMP ON THE CEREBRAL CORTEX \*

(ANIMAL EXPERIMENTS)

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## INTRODUCTION

Treatment by physical methods, especially by light, has been for some time a matter of common custom among clinicians; but recent progress of medical science has opened up new pathways in radiotherapy; especially have the Roentgen ray, radium ray, and ultraviolet ray been put on a more scientific foundation.

In general, they all have a destructive action on living cells, but each has its special characteristics; in therapy it is desirable to make the best use of these special characteristics.

I believe that up to the present there has not been an efficient study of the characteristics, actions and influences of the ultraviolet radiations. I determined to study their action on the cerebral cortex of rabbits. I employed to produce them, Kromayer's mercury quartz lamp, as the radiations from it consist of a very high percentage of ultraviolet rays and a low percentage of violet and blue rays.

1. *Kromayer's Mercury Quartz Lamp*.—The mercury quartz lamp is an outgrowth of the mercury vapor light, investigated by Arons in 1892, and found to give a light very rich in chemical rays, especially the ultraviolet; but these rays are absorbed by the glass, so that the light which passes through the glass tube has no pathologic influence on tissues.

Küch, therefore, made the apparatus with quartz instead of glass; for, not only can all the ultraviolet rays radiating from the mercury quartz lamp pass through, but there is a strong resistance for the high heat; but more or less heated light still passed through the apparatus, so Kromayer modified it in 1904, ridding it of the heated light by adding a cooling apparatus. So this apparatus is called Kromayer's mercury quartz lamp (*Kromayer'sche Quecksilberquartzlampe*).

2. *Actions of the Radiations from the Mercury Quartz Lamp*.—Because the radiations from the mercury quartz lamp have irritative and disinfecting actions, Kromayer reported in 1906 the experimental results of his study of the radiations. He noted (1) that the

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radiations caused inflammatory and disinfective action of the skin; (2) that they could be made to invade the deeper tissues; (3) that the strength of these actions was in direct proportion to the quantity of the electric current used to produce the radiations.

*A. Irritative Action of the Radiations.*—The irritative action of the radiations is inversely proportional to the distance; that is, when at a distance, only slight inflammation of the skin is produced; but when the apparatus is in pressure contact with the skin the result is disturbance of the deeper tissues (much of the radiation is absorbed by the blood, consequently when the apparatus is in pressure contact with the skin a local anemia is produced by the pressure, and the rays are not absorbed), and the degree of disturbance is in direct proportion also to the radiating time.

Thus the disturbances produced are in direct proportion to both time of radiation and quantity of current lost, and inversely proportional to the distance.

*B. Disinfective Action of the Radiations.*—Downes and Blunt, in 1877, were the first to investigate the disinfective action of the ultraviolet rays, and afterward Courmont and Nogier, in 1909, studied the action in detail.

In general, the sensitiveness of bacteria to the ultraviolet rays differs with the variety; according to the studies of Cernovodeau and Herni, the disinfective action of the radiations from the mercury quartz lamp on many bacteria are as follows:

(a) The water examined had 10,000 to 100,000 bacteria per 1 c.c. before it was radiated.

(b) The electric current used to produce the radiations was of 110 volts and the lost quantity was 3 amperes.

(c) The distance to the surface of the water from the apparatus was 20 cm.

(d) Time in seconds required to sterilize the water completely:

	Seconds		Seconds
Staphylococcus .....	5 to 10	Vibrio cholerae .....	10 to 15
B. typhosus .....	10 to 20	B. dysenteriae .....	10 to 20
B. coli-communis .....	15 to 20	Diplococcus pneumoniae .....	20 to 30
B. anthracis .....	20 to 30	B. tetani .....	20 to 60
B. subtilis .....	30 to 60		

*C. Chemical Action of the Radiations.*—The chemical action of the radiations is very strong, and according to von Behring's studies, 10 per cent. chinone-alcohol solution may be turned black in four minutes, and 20 per cent. vanilin-alcohol solution brown in six minutes; besides these, it has many other reactions.

*D. Sensitiveness of the Human Body to the Radiations.*—It is said that the sensitiveness of the human body to the radiations is indefinite, and it is impossible to determine just the fit quantity for use, as in the case of the Roentgen ray.

## EXPERIMENTAL RESEARCHES

My own experimental researches on the influence of the radiations, especially the ultraviolet from the mercury quartz lamp, on the cerebral cortex of rabbits, are as follows:

*A. The Methods.*—Rabbits only were used in these experiments. A selected part of the head was prepared, the hair cut, the skin sterilized with alcohol, and then incised aseptically, the periosteum stripped from the cranium, the bone trephined and a piece of bone removed. Sutures were taken first in the stripped-up periosteum and then in the skin. The wound was covered with collodion and left to heal for about two days before radiating. Care was taken not to wound the brain substance during the operation.

The trephine which I used was 0.8 cm. in diameter; the part trephined was one side of the occiput; the other side was preserved for the control; the radiation was that from the mercury quartz lamp, produced with 3 amperes of direct current; the radiating method was the direct, and the radiated time was 10, 20, 30 and 60 minutes, respectively.

*B. Macroscopic Changes Caused by the Radiations.*—The macroscopic changes caused by the radiations, using the foregoing methods, are as shown in the accompanying tables:

TABLE 1.—DATA OF EXPERIMENTS WITH THE RADIATIONS

No. of Experiment	Sex	Weight of Body, Gm.	Trephined Part	Radiating Method	Time of Each Radiation, Minutes	The Strength of Radiations
1	Female (pregnant).	2,585	Right occiput.	Direct....	10	The radiations caused by 3 amperes of the direct current with resistance No. 1 attached to Kromayer's mercury quartz lamp. The resistance was made in Japan.
2	Male.....	1,955	Left occiput...	Direct....	20	
3	Male.....	2,050	Right occiput.	Direct....	30	
4	Female.....	2,455	Right occiput.	Direct....	60	
5	Female (28 days old)	345	Right occiput.	Direct....	10	
6	Male (28 days old)	350	Not trephined.	Radiated on the right	30	
7*	Male.....	950	Right occiput.	Not radiated	..	
8*	Male.....	2,165	Not trephined.	Not radiated	..	

\* Control.

*C. Control Examinations.*—(Experiments 7 and 8.) As controls I used two rabbits in good health which were not radiated.

*EXPERIMENT 7.*—In this case I trephined the right occipital region, but did not radiate; the rabbit was killed with chloroform on the seventeenth day after the trephining, and examined for histologic changes in the part of the brain trephined; there were no changes.

*EXPERIMENT 8.*—In this case I did not trephine or radiate; the rabbit was killed with chloroform, and a histologic examination made of the occipital portion of the brain.

*D. The Macroscopic Appearances of the Trephined Part.*—At the trephined opening new bone developed from the periosteum, and the skin radiated was thickened more or less.

*E. The Histologic Study of the Tissues Radiated.*—Histologic study was made by the following methods: 1. Fixing Fluids: (a) 10 per cent. formaldehyd solution; (b) Müller's fluid. 2. Mounting Methods: (a) celloidin mounting;



TABLE 2.—DATA OF EXPERIMENT ON RADIATIONS

No. of Experiment	No. of Treatment	Time of Radiating, Minutes	Weight of Body Gm.	Remarks
1	1	10	2,575	No changes.
	5	10	2,455	Miscarried, five rabbits with four treatments (on the fifth day after the first treatment)
	6	10	2,330	At the radiated part, baldness began to appear; its area was as large as a nickel (after six treatments)
	7	10	2,550	Baldness was noted
	12	10	2,305	Regeneration of hair on the bald part began on the eighth day after the loss of hair had begun. Paralysis was noticed in the left forefoot after sixteen treatments (on the twenty-eighth day)
	17	10	2,440	Paralytic condition was noted
	18	10	2,475	Paralytic condition was noted
	24	10	2,550	Paralytic condition was noted
		0	2,370	Killed with chloroform on the thirty-seventh day after the first treatment
	—	—	—	—
	24	4 hrs.		
2	1	20	1,920	No changes
	3	20	2,025	Paralysis was noticed in the right forefoot after two treatments (on the third day)
	4	20	2,075	Paralytic condition noted
	7	20	2,035	The paralytic condition was the same; and at the radiated part, baldness began to appear and its area was as large as a nickel (after seven treatments)
	8	20	2,050	Baldness was noted
		0	2,045	Regeneration of hair on the bald part began on the ninth day after the loss of hair had begun; the paralytic condition was the same
		0	2,085	The paralytic condition was the same; animal killed with chloroform on the 32d day after first treatment
	—	—	—	—
	8	2 hrs. 40 min.		
3	1	30	2,080	No changes
	2	30	2,240	Paralysis was noticed in the left forefoot the twenty-second hour after the first treatment
	3	30	2,185	Paralytic condition was noted
	6	30	2,125	The paralytic condition was the same; and at the radiated part baldness began to appear and its area was as large as a nickel; the skin of this part was thickened notably (after six treatments)
		0	2,115	The paralytic condition was the same, and regeneration of hair on the bald part began on the seventh day
		0	2,115	The paralytic condition was the same; animal killed with chloroform on the thirty-first day after the first treatment
	—	—	—	—
	6	8 hrs.		
4	1	60	2,300	No changes
	2	60	2,470	Paralysis was noticed in the left forefoot the twentieth hour after the first treatment
		0	2,510	Paralytic condition noted; at the radiated part baldness began to appear on the sixth day after the first treatment; its size was the same as in No. 1
		0	2,515	The paralytic condition was the same; regeneration of hair on the bald part began on the seventh day
		0	2,530	The paralytic condition was the same; animal killed with chloroform on the twenty-first day after the first treatment
	—	—	—	—
	2	2 hrs.		

TABLE 2—Continued

No. of Experiment	No. of Treatment	Time of Radiating, Minutes	Weight of Body Gm.	Remarks
5	1	10	340	No changes
	2	10	350	Paralysis was noticed in the left forefoot the twenty-fourth hour after the first treatment
	3	10	370	Paralytic condition noted
	7	10	480	The paralytic condition was the same; at the radiated part baldness began to appear after six treatments (on the seventh day); its size was the same as in No. 1
	8	10	450	The paralytic condition was the same; at the radiated part baldness began to appear after six treatments (on the seventh day); its size was the same as in No. 1
		0	467	The paralytic condition was the same; regeneration of hair on the bald part began on the sixth day
		0	498	The paralytic condition was the same; animal killed with chloroform on the thirty-first day after the first treatment
	8	1 hr. 20 min.		
6	1	30	350	No changes
	7	30	500	No changes
		0	550	No changes; animal killed with chloroform on the thirty-first day after the first treatment
	7	3 hrs. 30 min.		

(b) paraffin mounting; (c) Fukushi's (Japanese) gelatin mounting; (d) freezing method. 3. Thickness of the sections, 5 or 6 microns.

4. Staining Methods: (a) hematoxylin-eosin double staining; (b) Lenhossek's staining for ganglion cells; (c) Nissl's staining for ganglion cells; (d) Bielschowsky's staining; (e) Marchi's staining; (f) Taumura's (Japanese) staining for the neurilemma and axis-cylinder; (g) fat staining with sudan III.

#### MICROSCOPICAL EXAMINATIONS

A. Histologic examination of the radiated part of the cerebral cortex (Experiments 1 to 6).

##### EXPERIMENT 1:

##### 1. The molecular layer.

There were seen no pathologic changes.

##### 2. The layer of small pyramidal cells.

In general, the nerve cells of the superficial part of this layer were atrophied, and the cells of the deeper part close to the layer of the large pyramidal cells were swollen. Most of the swollen cells showed a perinuclear chromatolysis and vacuolation of the cytoplasm at their periphery; some cells burst because of the great swelling; some showed a displacement of the nucleus; some showed the phenomena of karyolysis, karyorhexis and pyknosis of the nuclei. In general, those cells which showed karyolysis and those which were destroyed, reacted very weakly to the staining solutions. Such phenomena as chromatolysis, karyolysis, karyorhexis, displacement of the nucleus, vacuolation and swelling of nerve cells were very marked in the deeper parts; in the superficial parts there was seen only contraction.

3. { The layer of large pyramidal cells.
- { The layer of polymorphous nerve cells.

\*The pathologic changes in both of these layers generally resembled the

changes in the layer of small pyramidal cells, but their degree was much greater than in the second layer. In these four layers there was seen no fatty degeneration of the nerve cells or any degeneration of the neurofibrils, but a slight infiltration of small round cells.

#### EXPERIMENT 2:

1. The molecular layer.

There were seen no pathologic changes.

2. The layer of small pyramidal cells.

The pathologic changes in the nerve cells of this layer generally resembled those of the second layer of Experiment 1, but in lesser degree. The nerve cells of the superficial part were atrophied less than those of Experiment 1; those of the deeper part were in general swollen and the nuclei were degenerated, but the vacuolation was slight.

3. { The layer of large pyramidal cells.  
The layer of polymorphous nerve cells.

The changes in both layers resembled the changes in the deeper part of the layer of small pyramidal cells of Experiment 1; but in this case (1) there were seen many nerve cells with less protoplasm, because the nucleus was notably swollen; and (2) in other cells the nuclei were destroyed and the bodies of the cells vacuolated; in this case, also, the pathologic changes in the nerve cells were greater in the deeper part than in the superficial part. In all layers there were seen no fatty degeneration of the nerve cells or any degeneration of the neurofibrils, but a slight infiltration of small round cells.

#### EXPERIMENT 3:

1. The molecular layer.

There were seen no pathologic changes.

2. { The layer of small pyramidal cells.
3. { The layer of large pyramidal cells.
4. { The layer of polymorphous nerve cells.

In general, the pathologic changes of these three layers resembled the changes of those of Experiment 1, but much greater in degree; for example, many nerve cells were atrophied, especially in the layer of small pyramidal cells; the number of swollen cells was increased in the deeper part and the cell bodies were notably vacuolated. The nuclei of some of the vacuolated cells was already destroyed. In some of the swollen cells at the deeper part the volume of protoplasm, because of the swollen nuclei, was greatly diminished. In general, the superficial cells were atrophied, while the deep cells were swollen and vacuolated. In none of the layers was there seen any fatty degeneration of the nerve cells or any degeneration of the neurofibrils, but a slight infiltration of small round cells.

#### EXPERIMENT 4:

1. The molecular layer.

There were seen no pathologic changes.

2. { The layer of small pyramidal cells.
3. { The layer of large pyramidal cells.
4. { The layer of polymorphous nerve cells.

In general, the pathologic changes in the nerve cells in these three layers resembled, but were less than, the changes of those of Experiment 3. The nerve cells of these three layers were generally swollen and some of them vacuolated, and also some of the cells of the first layer were atrophied. The changes of the cytoplasm and nucleus were the same as those of Experiment 3.

In this case, also, in none of the layers was there seen any fatty degeneration of the nerve cells or any degeneration of the neurofibrils, but a slight infiltration of small round cells.



## EXPERIMENT 5:

## 1. The molecular layer.

There were seen no pathologic changes.

## 2. { The layer of small pyramidal cells.

## 3. { The layer of large pyramidal cells.

## 4. { The layer of polymorphous nerve cells.

The pathologic changes of the nerve cells in these three layers were about the same as those of Experiment 4, but in this case the nerve cells were swollen only in the layer of polymorphous nerve cells; in the layer of small pyramidal nerve cells there was more or less atrophy. The vacuolation of the cytoplasm was seen in the third and fourth layers, and in a very few instances in the second layer; also there were seen a few cells which were already destroyed and which showed a diffuse, homogeneous staining of their bodies.

In none of the layers was there seen any fatty degeneration of the nerve cells or any degeneration of the neurofibrils, but a slight infiltration of small round cells. In short, in this case, the degree of the pathologic changes is moderate.

## EXPERIMENT 6:

## 1. The molecular layer.

There were seen no pathologic changes.

## 2. { The layer of small pyramidal cells.

## 3. { The layer of large pyramidal cells.

## 4. { The layer of polymorphous nerve cells.

Some of the nerve cells in these three layers were swollen and chromatolyzed more or less, but the degree of the changes was much less than in those noted previously; and none of the other changes was recognized.

## B. Histologic examination of the control part (Experiments 1 to 6).

In none of these layers were there found any pathologic changes.

## EXPERIMENT 7: (Control examination.)

In the histologic examination of the trephined and control parts there were found no pathologic changes in either.

## EXPERIMENT 8: (Control examination.)

In this case there was no change, because the animal was in good health and used only for the control examination.

## SUMMARY

Following is a summary of facts found in the foregoing experiments.

1. The radiations used for the experiment were produced from Kromayer's mercury quartz lamp, 3 amperes of the direct current being used, with resistance No. 1 attached to the apparatus.

2. The strength of the radiations was the same in all cases.

3. The radiating method was "the direct" in each case.

4. The results of Experiment 1 were: miscarriage after four treatments (forty minutes) on the fifth day; paralysis of the left fore foot after sixteen treatments (two hours and forty minutes) on the twenty-eighth day; the degree of the paralysis became greater with each treatment. As to the microscopic pictures of the cerebral cortex, in general, the nerve cells in the superficial part were atrophied, but the cells in the deeper part were swollen and presented the phenomena of chromat-

olysis, displacement of the nucleus, karyolysis, karyorhexis and vacuolation.

5. In Experiment 2 the results were: paralysis of the right fore foot after two treatments (forty minutes) on the third day. The degree of paralysis became greater with each treatment. As to the microscopic pictures of the radiated part of the cerebral cortex, they resembled those of Experiment 1, but were less in degree.

6. In Experiment 3 the results were: paralysis of the left fore foot after two treatments (one hour); this condition was noticed the twenty-second hour after the first radiation, and the degree of the condition became greater with each treatment. As to the microscopic pictures of the radiated part of the cerebral cortex, they resembled those of Experiment 1, but the changes were more marked.

7. In Experiment 4 the results were: paralysis of the left fore foot after two treatments (two hours); this condition was noticed the twentieth hour after the first radiation. The microscopic pictures of the radiated part of the cerebral cortex resembled those of Experiment 3, but were less in degree.

8. In Experiment 5 the results were: paralysis of the left fore foot with two treatments (twenty minutes), and this condition was noticed the twenty-fourth hour after the first radiation; the degree of the paralysis became greater with each treatment. The microscopic pictures of the radiated part of the cerebral cortex resembled those in Experiment 4, but the changes were more marked.

9. In Experiment 6 the rabbit was only 23 days old and was radiated without trephining for purposes of control. Macroscopically, there were no changes; as to the microscopic pictures of the radiated part of the cerebral cortex, in general, the nerve cells throughout the second to the fourth layers were swollen more or less, and a few of them presented the phenomena of chromatolysis.

10. Experiment 7 was performed to determine whether or not paralysis of the foot would result from trephining without radiating, as a control examination; there were recognized no macroscopic or microscopic changes.

11. In Experiment 8 a rabbit in good health, without trephining or radiation, was used for the microscopic examination of the occipital cortex as a control.

12. In the tissues of the control parts in Experiments 1 to 6 there were no changes.

13. Surface effect at the radiated part: Baldness was noticed about seven days from the first radiation, but there was a regeneration of

hair about seven days later (in Experiments 1 to 5); in Experiments 6 and 7 there was not such a process.

14. In the trephined part new bone developed from the periosteum; the radiated skin was thickened more or less.

15. The weight of the body during the experiment in each instance was almost without change.

#### CONCLUSIONS

While it is generally believed that the action of the Roentgen ray on the central nervous system is very weak, the action of the radiations from Kromayer's mercury quartz lamp is remarkably strong, and the effects are due to the ultraviolet rays, which are the chief constituents of the radiations. The effect of the radiations on the cerebral cortex of the rabbits experimented on was as follows:

1. The strength of the action of the radiations is in proportion to the time of radiation.

2. The effect of the radiations on the cerebral cortex is the disturbance of the nerve cells as heretofore mentioned; that is, the production of swelling, atrophy, chromatolysis, karyolysis, karyorhexis, displacement of nucleus, vacuolation and cell death.

3. As shown by Experiments 1 to 5, the retrogressive processes in the nerve cells begin, at first, with the superficial part of the cerebral cortex, and gradually progress into the deeper part.

4. The resistance of the cerebral cortex of the young rabbit against the radiations is very much weaker than in case of old rabbits, as shown in Experiments 5 and 6.

5. The paralytic condition of the fore foot of the experimented animals is due to the results of the retrogressive processes in the nerve cells.

6. No pathologic changes in the neurofibrils could be recognized, even after the nerve cells had notably degenerated, but it is believed that the neurofibrils suffer some degenerations on account of the retrogressive processes in the nerve cells.

7. The radiations may more or less pass through the cranium of the very young rabbit, as indicated by Experiment 7.

8. It is impossible to determine just what quantity of radiations is needed for proper use, as in the case of Roentgen rays.

9. In Experiments 1 to 5 the baldness produced at the radiated part of the experimented animals was due, it is believed, to the temporary disturbances of nutrition of the skin, because of the failure of the skin to unite with the surrounding tissues over the trephine wound, and also because of the pressure on the blood vessels at that part by



the Kromayer apparatus. The process of regeneration of hair which followed on the bald spot was due to the irritative action of the radiations.

10. The thickening of the skin at the radiated part is due to the chronic irritative action of the radiations.

This work was carried out at the laboratory of St. Luke's Hospital, Tokio, Japan, in September, 1915, and it is a great pleasure to express to Dr. Y. Kon, professor of pathology at the Tokio Charity Hospital Medical College, my sincerest thanks for his many helpful suggestions and aid given to me in carrying out the details of this study; and also to express my gratitude to Dr. E. Iida, head of the department of dermatology at St. Luke's Hospital, for permission to use the Kromayer mercury quartz lamp.

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## THE REFLEX ACTION OF VOLATILE IRRITANTS ON THE CIRCULATION \*

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Among the measures in common use in combating conditions of threatened or actual circulatory failure, is the subcutaneous or intramuscular injection of volatile substances of a highly irritant nature. Among these so-called circulatory stimulants are ether, alcohol in the form of whisky or brandy, and camphor dissolved in ether, alcohol, or oil. The clinical use of these substances in shock and allied conditions is based, not on exact experimental observation, but on casual bedside impression and tradition.

### OBJECT OF THE EXPERIMENTS

As a result both of laboratory observation and clinical experience, we have for some time held the opinion that the effects of the injection of volatile irritants were due, not to a direct stimulation of the circulation, but to a reflex action arising from intense irritation of sensory nerve endings. That intense irritation or stimulation of a sensory nerve may lead to a reflex rise in blood pressure has been known for a long time, but as far as we have been able to ascertain from the literature, this explanation of the action of volatile irritants is not the one ordinarily offered. If this group of substances acts in the manner suggested, the importance of a critical study of measures so universally used by clinicians becomes at once apparent. Any change in the circulation following the employment of these drugs is brought about by excitation of the medullary centers by afferent impulses, initiated at the site of injection. A rise in blood pressure presupposes a vasomotor center which is capable of excitation. These drugs are used almost exclusively in the treatment of shock and collapse — conditions in which the center is believed either to be paralyzed, and therefore incapable of excitation, or to be so active that further stimulation of the center does not bring about any large increase of blood pressure. In either case bombardment of the vasomotor center by afferent stimuli will result in no material increase in blood pressure. Furthermore, the pain at the site of injection may lead to a deepening of the shock or collapse already present.

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## EXPERIMENTAL STUDY OF ACTION OF VOLATILE IRRITANTS

*Technic.*—All the experiments were performed on cats, decerebrated under ether, the temperature of the animal being maintained by an electric pad. An interval of at least twenty minutes was allowed to elapse between decerebration and the beginning of the experimental record, so that the ether was completely eliminated and reflex irritability restored. Such an animal is incapable of feeling pain. It breathes spontaneously, but in effect is nothing but a reflex machine.

It is worthy of note that the reflex irritability of an animal is greatly increased by destruction of the cerebral hemispheres. Hence the response to a given stimulus is much more pronounced in decerebrate animals than in normal individuals.

TABLE 1.—DATA OF—

Substance	Abdomen	Chest Wall	Leg (Thigh)	Hand
Ringer's solution.....	I* 2	I 0	.....	II 0
Water.....	I 1	I 0	II 3.5	II 10
Alcohol.....	I 0	I 0	I 0	VII 47.3
Ether.....	I 0	.....	I 0	VIII 17.6
Camphor and ether.....	I 0	II 4	II 4	XI 9.8
Turpentine.....	III 0	II 5	II 0.5	XL 8.1
Camphor and oil.....	.....	II 0	II 0	III 13.7
Total injections.....	VIII	IX	X	LXXIII
Average rise in blood pressure..	0.4	2	1.6	10.3

\* Roman numerals indicate the number of injections. Arabic numerals indicate the

For the injections, a glass syringe equipped with a 28-gage needle was used. A needle of such small caliber causes a minimum of local trauma—a factor of importance in estimating the reflex irritant effect of the substance introduced into the tissues through the needle. As a further precaution, the effect on blood pressure of the introduction of the needle alone was observed and an interval of several seconds allowed to elapse between the penetration of the tissues by the needle and the injection of the irritant. In many instances in which the animal was more than ordinarily irritable, the puncture of the skin by the small needle was followed by a rise in blood pressure, or by convulsive movements. These effects were similar to, though less than, those brought about by the irritant injections themselves.

*Substances Used.*—These were the usual volatile irritants employed in clinical work; ether; camphor, 10 per cent. in ether; camphor, 20 per cent. in oil; "spirits of camphor," (camphor, 10 per cent. in alcohol); whisky; and in addition, oil of turpentine. The latter was chosen because it is an intense irritant without immediate grave toxic effects. Ringer's solution, distilled water and tincture of digitalis also were used.

## TABULATED RESULTS

The results are presented in tabular form in order to avoid detailed report of protocols. These tables are arranged to show the effect on blood pressure of: (1) the substance injected; (2) the site of injection (3) the comparative effects of the different volatile irritants.



In compiling these tables from the protocols, it is realized that comparison of quantitative results is not strictly allowable, since the experimental conditions were so variable. However, only those experiments are used in which conditions were usual—the animal having intact reflexes, not having suffered impairment of the central nervous system through asphyxia, faulty technic in decerebration, etc. As the total number of injections was large, the average figures have some value, however inexact.

—AUTHOR'S EXPERIMENTS

Foot	Nose	Dorsum of Foot	Muscle	Arm	Total Injections and Average Rise in Blood Pressure
I 0	.....	.....	.....	I 0	VI 0.5
.....	.....	.....	.....	.....	VI 3.6
II 1	.....	.....	.....	.....	XII 3.6
IX 5	I 28	.....	.....	.....	XX 10.1
XIX 18.5	I 21	I 34	.....	.....	XXXVII 13.0
XXXI 9.9	II 12.5	I 18	I 7	.....	LXXXII 7.6
I 8	.....	.....	.....	.....	VIII 5.3
LXIII	IV	II	I	I	
11.8	18.5	26	7	0	

average rise in blood pressure.

SITES OF INJECTION

Reference to Table 1 shows that irritants were injected under the skin of the following regions: pads of the hands and of the feet, thighs, forearm, abdomen and chest wall. A number of injections were also made into the submucous tissue of the nasal septum. Of these regions the submucosa of the nose seemed to be the most irritable, since the reflex rise in blood pressure was the greatest. That this rise in blood pressure was not due to partial asphyxia as a result of the manipulation, is proved by the fact that after tracheotomy similar increase in blood pressure occurred on injection into the nasal submucosa.

Next in degree of sensibility are the pads of the hands and feet. When injections are made under or into the pads, a prompt and considerable rise in pressure usually occurs. It is interesting to note that the rise in blood pressure following injection into the pads of the feet, is somewhat greater than that following injections of similar doses into the pads of the hands.

The least sensitive areas seem to be the subcutaneous tissue of the

general body surface, for injection into these tissues results in practically no change in blood pressure. In this connection it may be pointed out that decerebrated animals are incapable of appreciating pain, hence changes in blood pressure following the injection of irritant substances are due solely to the reflex action on the vasomotor centers. On the other hand, in intact, unanesthetized animals, such injections are extremely painful and rise in pressure may occur as a result of this disturbance of consciousness.

It may be permissible to conclude from these experiments that the order of sensibility of the various regions injected is as follows: (1) nasal septum; (2) pads of feet; (3) pads of hands; (4) the general body surface. These results are in accord with the observations of von Frey<sup>1</sup> and of Crile<sup>2</sup> on the comparative sensibility of various parts of the body surface.

#### RELATIVE IRRITABILITY OF THE VARIOUS SUBSTANCES USED

The irritating character of a substance depends partly on its volatility, partly on osmotic tension, its ability to coagulate protein, and on other inherent and less well defined qualities. Ether, alcohol, and turpentine are volatile substances and seem irritant in proportion to their volatility. The irritant character of ether may be enhanced by having camphor in solution. Oil is by no means a bland substance when brought into direct contact with the sensory nerve-endings in the skin. Camphor in solution doubtless increases the irritant effects of the oil. Water is irritant largely through its alteration of the osmotic relations of the parts injected. Aside from other physical factors, all subcutaneous injections are painful by reason of the local tension brought about. In an injection of a fluid like Ringer's solution, only the last named factor comes into play. Judging from the degree of response, in the way of rise in blood pressure, from subcutaneous injection of the substances used, the order of irritability is as follows: Ringer's solution, practically non-irritant; distilled water, alcohol, equally irritant; camphor in oil, turpentine, ether, camphor in ether. (See Table 1.)

#### DURATION OF EFFECT

The total duration of the response to the injection was usually less than fifteen minutes. A typical reaction is shown in Figure 1. At the first signal the needle of the hypodermic was plunged through the skin

1. Von Frey: Starling, "Human Physiology," 1915, p. 490.

2. Crile, G. W.: "Surgical Shock," Philadelphia, 1899, p. 122, et seq.

covering the center pad of the right foot. Three seconds later, at the second signal, 1 c.c. of oil of turpentine was injected. A few seconds after the injection was begun blood pressure began to rise. The maximum rise, which was about 16 mm. of mercury, was reached in three seconds and was followed by a *quick* fall of about 10 mm. At this point blood pressure was still 6 or 8 mm. above normal. The pressure now fell more gradually, so that the normal pressure was regained twelve minutes after injection. In a small percentage of the experiments the rise in blood pressure was followed by an abrupt fall to normal, so that the entire reaction lasted less than two minutes. (See Figure 3, A, experiment of Dec. 3, 1915.) The extreme transientness of the complete cycle suggests that the therapeutic use of these substances is very limited.

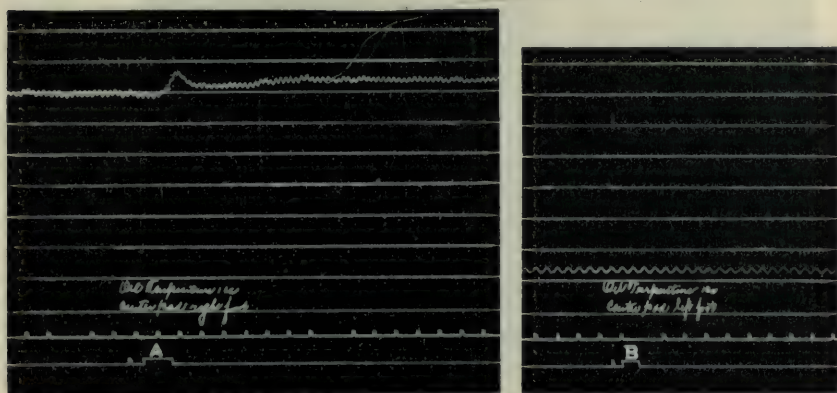


Fig. 1.—Cat decerebrated under ether. At A, 1 c.c. of oil of turpentine was injected into the center pad of the right foot; nerve supply intact. At B, 1 c.c. of oil of turpentine was injected into the center pad of the left foot; entire central nervous system had been destroyed. In this and the subsequent tracings the lowest line serves as a signal; the next above gives time in five-second intervals, the third line from the bottom corresponds to the zero of blood pressure.

#### RATE OF INJECTION

The effect on blood pressure seems to be directly proportional to the intensity of the irritation. Figure 2, experiment of Dec. 29, 1915, shows at B the effect of the injection of 1 c.c. of oil of turpentine into the center pad of the right hand. The time occupied by the injection was approximately three seconds. The first part of the tracing at A shows the effect on blood pressure when the same amount of oil was injected into the corresponding pad of the other hand. The only difference in technic is the time required to complete the injection. Injection B occupied approximately three seconds, whereas Injection A required 110 seconds. In one case, irritation is sudden and sharp and is followed by a profound change in blood pressure; in the other, irritation is mild and prolonged and causes no rise in blood pressure.



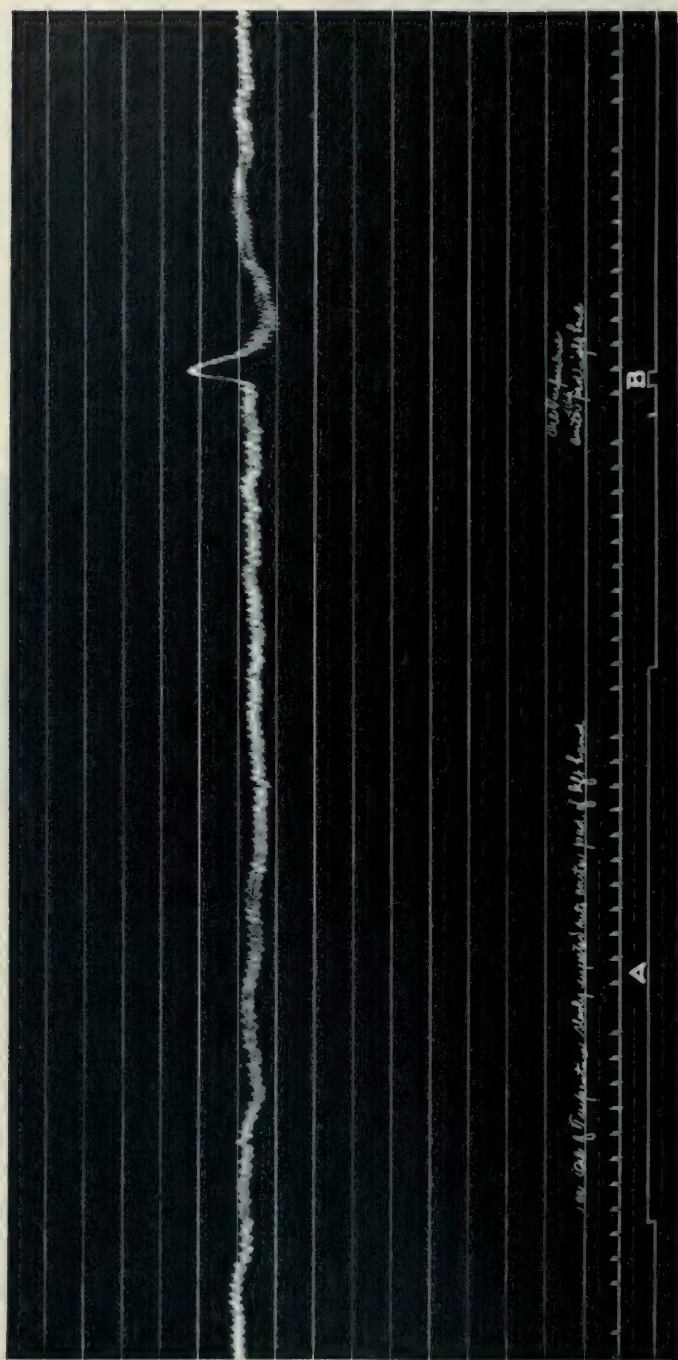


Fig. 2.—Cat decerebrated under ether. At A, 1 c.c. of oil of turpentine was injected into the center pad of the left hand; time required to complete injection, 110 seconds. At B, 1 c.c. of oil of turpentine was injected into the center pad of the right hand. Injection time, three seconds.

## REPEATED INJECTIONS

In some of the earlier experiments, in which camphor and ether were injected at short intervals, it was found that there was throughout the experiment a gradual and progressive fall of the average blood pressure. At the same time, successive doses of camphor and ether produced relatively smaller and smaller increases in blood pressure. To account for this a number of explanations suggested themselves. In the first place, the effects might be due to the gradual paralysis of the vasomotor center by the progressive accumulation of ether; or they might be the result of exhaustion of the vasomotor center from repeated afferent stimulation; in other words, we might be dealing with a form of traumatic shock; or, finally, the repeated excitation of the same nerve paths might result in their exhaustion. The direct paralytic action of the irritants on nerve-endings was excluded, since no two injections were made into exactly the same site. That the gradual depression of blood pressure was the result of the accumulation of ether was shown by the experiment of Dec. 14, 1915.

Successive doses of 1 c.c. of camphor and ether were injected into different pads of the feet and hands. The first injection was followed by a rise in blood pressure of 12 mm.; the second, five minutes later, resulted in a rise of 10 mm.; the third, five minutes later, gave a rise of 20 mm.; the fourth, after a five-minute interval, gave a rise of 8 mm.; the fifth injection, six minutes later, gave no rise; neither did the sixth, seven minutes later; nor the seventh, five minutes later. In two experiments of Dec. 17, 1915, in which ether 1 c.c. was injected at intervals of ten minutes, thus allowing time for the elimination of ether, the blood-pressure response to the later injections was not lessened.

In other words, if ten minutes be allowed to elapse between injections of 1 to 2 c.c. of ether, no progressive fall in blood pressure occurs. If injections are repeated at two minute intervals, ether accumulates in the organism and prevents reflex blood pressure response.

Because of the danger of ether's depression of the central nervous system and the consequent masking or abolition of reflex effects, recourse was had to oil of turpentine. This volatile oil is highly irritant and causes stimulation of the medulla and spinal cord. Hence, one might expect not a progressive loss, but a progressive increase of irritability, resulting eventually in convulsions. With this substance, repeated injections led to no marked permanent fall in blood pressure, and no marked change in the degree of the vasomotor response to repeated injections until convulsions developed.

## SHOCK FROM REPEATED INJECTIONS

The possibility of inducing shock by the repeated traumatism of irritant injections was studied in a few instances. Oil of turpentine was chosen because its toxic effects are comparatively slow in appearing, while the irritant effects are marked and immediate. Collapse could

not be induced before the convulsions of the toxic stage of turpentine action appeared.

In the experiment of Feb. 1, 1916, 23 injections of oil of turpentine, 1 c.c. each, were given in the course of the experiment. Injections 1 to 9, inclusive, were followed by rise in blood pressure varying from 20 to 2 mm. Injections 20 to 23, inclusive, were not followed by any rise in blood pressure. A terminal asphyxial rise of blood pressure showed that the vasomotor center was not paralyzed. Beginning with Injection 15, there were signs of turpentine poisoning, namely, vomiting and convulsive movements. No signs of collapse appeared in this animal.

#### THE EFFECT OF SUCCESSIVE INJECTIONS INTO THE SAME REGIONS

This phase of the study involves the question of the effect on the nerve-endings of the site of injection, and the reflex arc. A short though variable time, usually not less than two minutes, is required for the recovery of a degree of irritability of the nerve-endings at the site of injection.

TABLE 2.—EXPERIMENT OF DECEMBER 17: INJECTION OF 1 C.C. CAMPHOR 10 PER CENT. IN ETHER

Number of Injection	Time of Injection	Site	Change in Blood Pressure, Mm.
I	2:35	Second pad of right foot.....	+ 20
III	3:15	Second pad of right foot.....	+ 20
II	2:55	Third pad of right foot.....	+ 18
IV	3:30	Third pad of right foot.....	+ 10
V	3:33	Center pad of right foot.....	+ 11
VI	3:35	Center pad of right foot.....	+ 10

It will be noted that in the experiment of December 17 there was exhaustion neither of the nerve endings nor of the reflex arc from successive injections into the same site or sites involving approximately the same reflex arc. The recovery of irritability of nerve endings is shown to be prompt and to take place in less than two minutes (see Injections V and VI).

In several similar experiments with different irritants, corresponding results were obtained.

#### THE NATURE OF THE RESPONSE

Our original hypothesis was that the reaction to the subcutaneous injection of an irritant is reflex in origin. The impulses arise at the point of injection, pass along the afferent nerves to the central nervous system and excite the vasomotor center. If this assumption is correct, any procedure which interrupts this arc will prevent the development of the reflex rise in blood pressure.



1. *Cutting of the Afferent Nerves.*—Section of sensory nerves prevents the usual rise in blood pressure when an irritant is injected into the denervated area.

In the experiment recorded in Figure 3, at A, 1 c.c. oil of turpentine was injected into a pad of the left foot. There was an immediate rise of blood pressure of 38 mm. A similar injection into the central pad of the right foot also caused a rise of 38 mm. The left sciatic and crural nerves were then cut. Five minutes later at B, Figure 3, 1 c.c. of oil of turpentine was injected into a foot pad of the denervated left leg. Blood pressure remained constant at the previous level.

This experiment shows that when afferent impulses are prevented from reaching the vasomotor center the injection of volatile irritants fails to cause a rise in blood pressure. The fact that the injection into the left leg caused the usual response, indicated that the vasomotor center was active and capable of stimulation. Like results were obtained in a number of experiments.

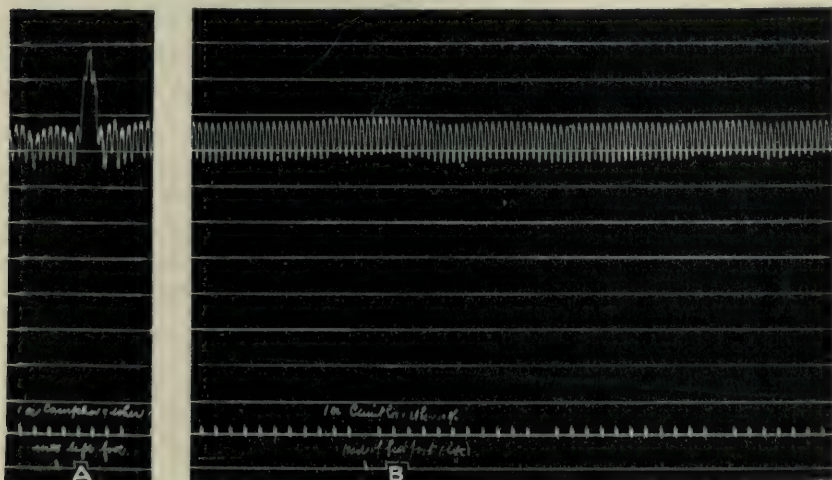


Fig. 3.—Cat decerebrated under ether. At A, 1 c.c. of camphor and ether (10 per cent.) was injected into a pad of the left foot; nerve supply intact. At B, 1 c.c. of camphor and ether (10 per cent.) was injected into another pad of the left foot; sciatic and anterior crural nerves had been cut.

2. *Depression of the Vasomotor Center.*—In a number of experiments ether was administered by artificial respiration until decerebrate rigidity disappeared. Under these conditions the subcutaneous administration of volatile irritants failed to cause a rise in blood pressure. The same absence of blood-pressure response to injection of irritants occurred when the vasomotor center was injured by a faulty technic in decerebration.

3. *Destruction of the Central Nervous System.*—In three experiments the vasomotor center was destroyed by pithing. In these animals the subcutaneous injection always failed to cause a rise in blood pressure. This is well shown in the experiment of April 5, 1916. The cat was decerebrated under ether as usual. The injection of 1 c.c. of oil of turpentine into the center pad of the right foot caused a rise of blood pressure of 18 mm., from 138 mm. to 156 mm (Fig. 1, A). The medulla was then destroyed. Two minutes later the blood pressure was 27 mm. The injection of oil of turpentine into the center pad of the left foot failed to modify the blood pressure in the slightest degree (Fig. 1, B).

4. *Intravenous Injections.*—Oil of turpentine was injected intravenously in several animals; 1 c.c. of the pure oil caused a prompt fall in blood pressure and usually resulted fatally. Smaller doses almost invariably caused a fall in blood pressure, never a rise. Thus in the experiment of April 3, 1916, 1 c.c. of an official emulsion of oil of turpentine caused the blood pressure to fall from 160 mm. to 80 mm.

That the heart plays little or no part in the rise in blood pressure following the subcutaneous injection of irritants was evident from those experiments in which the heart's movements were recorded by the Cushny myocardiograph. In these experiments neither the systole nor the diastole of the heart was changed; the rate remained practically constant.

#### EFFECT OF INJECTION OF IRRITANTS IN SEVERE HEMORRHAGE AND COLLAPSE

In just those conditions in which the measures studied are commonly used, they are least effective. In hemorrhage with falling blood pressure the subcutaneous injection of irritants is of little avail in checking the fall.

In the experiment of Dec. 10, 1915, three injections of camphor and ether, 1 c.c. each, into the pads of the feet, increased blood pressure 36, 42 and 42 mm., respectively. The animal was then bled, with resulting fall of blood pressure from 180 mm. to 100 mm. Camphor and ether injected into the pad of the right hand two minutes later gave no response. Three minutes later a similar injection into the pad of the left hand gave no response. The viscera were then manipulated and the pregnant uterus excised. Camphor and ether, 1 c.c., injected into the pad of the left hand gave no rise of blood pressure.

In shock, induced by evisceration, with exposure of the viscera to cold, severe manipulation and traction on the mesentery, nephrectomy, etc., the irritants have little or no influence in checking fall of blood pressure.

In the experiment of Dec. 29, 1915, the abdomen was opened, the viscera exposed, cold water poured on them and the mesentery stretched. Four minutes after this manipulation general blood pressure fell from 190 mm. to 120 mm. One c.c. of oil of turpentine was injected into the center pad of the right foot. The blood pressure rose 20 mm. Later both kidneys were removed, and manipulation of the liver and diaphragm carried out. The blood pressure rose 40 mm. and then fell 40 mm. Oil of turpentine, 1 c.c., was injected into the second pad of the left hand. Blood pressure rose 3 mm., to 95 mm., then gradually fell to 78 mm. Blood pressure continued to fall, and when it reached 40 mm. oil of turpentine was injected into the second pad of the right hand, with no effect. Another injection into the center pad of the left foot also had no effect. Blood pressure continued to fall, and the animal died five minutes later.

Experiment of Dec. 28, 1916: Several injections of irritants into the pads of feet and hands gave the usual rises in blood pressure. The abdomen was opened, the viscera roughly handled and cold water poured into the abdominal cavity. During these procedures blood pressure fell 70 mm., to 120 mm. Oil of turpentine, 1 c.c., injected into the third pad of the left hand, was followed by slight rise in blood pressure, 7 mm. Five minutes later, oil of turpentine, 1 c.c., injected into the third pad of the right hand produced no effect.



## DISCUSSION OF RESULTS

In a series of experiments in which the volatile irritants ordinarily used in clinical work and, in addition, oil of turpentine, were injected into decerebrate animals, no direct action on the circulation could be observed other than threatened or actual collapse when the irritants were administered intravenously. Subcutaneous injections caused a rise of blood pressure only when the reflex arc was anatomically and physiologically intact. Gross destruction of the central nervous system or severance of nerve supply of the part injected annuls the circulatory effect of the measures studied. Deep anesthesia (ether), severe hemorrhage, shock and collapse likewise abolish the blood-pressure-raising effect of volatile irritants. In other words, any measure destroying or profoundly depressing the function of any portion of the involved reflex arc robs the subcutaneous injection of volatile irritants of its blood-pressure-raising effect. Other points of interest are that the response is directly proportional to the irritant character of the substance administered and to the sensibility of the area injected.

That the rise in blood pressure following the subcutaneous administration of the volatile irritants is entirely reflex in character is a conclusion that in our opinion cannot be escaped.

The effect on blood pressure of the hypodermic administration of irritants, when the reflex arc is intact, is not great. When one takes into account the fact that the animals were made more than ordinarily irritable by decerebration, the blood pressure changes one might expect in animals with normal reflex irritability are very slight indeed, although in the event of retained sensibility the intense pain of an irritant might well modify the results. The transience of the circulatory effect of the irritant injections is important, the average duration of the rise being seconds. The blood pressure curve following these injections resembles that produced by pinching a sensitive part of the body surface, and is, in our judgment, but a sequel of a not very different local trauma. A series of irritant injections is generally followed by a fall in the average level of blood pressure. It is impossible to know whether or not such a fall is due to repeated bombardment of the central nervous system by painful afferent impulses, or to the toxic action of the substances used, or to other conditions of the experiment. It is impossible to produce traumatic shock by the subcutaneous injection of irritants, because of the early development of systemic toxic effects.

The bearing of the results of this study on the ordinary clinical use of the irritants is obvious. In circulatory deficiencies, the result of traumatic or toxic insult to the central nervous system, an irritant injection may fairly be called an added burden and may well accelerate oncoming shock. It is not claimed that the injection of irritants has no



place in clinical work. In conditions in which cutaneous sensibility is present and reflex irritability preserved, an irritant injection may rouse flagging energies for a momentary emergency. It is, however, a two-edged sword and may also be the final instrument of a collapse. The use of such a measure to stimulate an anesthetized or profoundly prostrated or unconscious patient has no experimental justification.

#### CONCLUSIONS

The volatile irritants are not direct vasomotor stimulants. They act reflexly through irritation of sensory nerve-endings.

Their effect on blood pressure is both slight and transient.

In conditions of abolished reflex irritability, as anesthesia, shock, etc., the volatile irritants are without effect on the blood pressure.

Their clinical value is questionable.

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## CLINICAL CALORIMETRY

NINETEENTH PAPER \*

### THE BASAL METABOLISM OF OLD MEN \*

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WITH THE TECHNICAL ASSISTANCE OF G. F. SODERSTROM

NEW YORK

The question of the basal metabolism at various ages has been discussed in Paper 12 of this series, and the references to the literature have already been published. It was apparent that our knowledge of the energy exchanges in old age depended almost entirely on the careful work of Magnus-Levy and Falk<sup>1</sup> who studied five old men and seven old women. These results were used by us in drawing the curves which indicated the average level of metabolism at various ages, but it was apparent that after the age of 43 the points on the chart were scanty. This present work is an attempt to fill in the gap.

An effort was made to find men 90 to 100 years old, but it was impossible to obtain suitable subjects. The age limits were then reduced to between 75 and 85 years, and, after much labor, six men were obtained who fulfilled the requirements. They were either inmates of the New York City Home for Aged and Infirm on Blackwell's Island, or men from other institutions about to be transferred to this home. They were all in good condition and fairly well nourished, though on plain and somewhat scanty diets. Considering their ages, they were in good health, though most of them suffered from arteriosclerosis, chronic interstitial nephritis and emphysema, which "normally" accompany advanced years. They were about as active as most persons of their time of life, none of them being engaged in regular work.

The men were selected by one of the writers from the somewhat limited number over 75 years of age who were in the City Home or Municipal Lodging House. Only those in fair health, with clear and apparently reliable histories as to their ages, were chosen, and an effort

\* Submitted for publication Dec. 19, 1916.

\* Clinical Calorimetry, Eighteenth Paper, The Number of Places of Significant Figures in the Data of Metabolism Experiments, by Gephart, Du Bois and Lusk was published in the Journal of Biological Chemistry, 1916, **27**, 217.

\* From the Russell Sage Institute of Pathology, in affiliation with the Second Medical Division, Bellevue Hospital.

1. Magnus-Levy and Falk: Der Lungengaswechsel des Menschen in verschiedenen Alterstufen, Arch. Anat. u. Physiol., 1899, Suppl., p. 315.

was made to pick out men who would cooperate in the experimental procedure. Most of them were delighted with the change of food and scene when they were taken from the almshouse to the metabolism ward. All the experiments were made in the morning, the men being placed in the calorimeter with no breakfast except a cup of "caffeine-free" coffee. The old men were about as quiet as the younger controls, with the exception of one whose test had to be repeated.

#### CASE HISTORIES

**CASE 1.—History.**—Andrew O'C., 77 years old, formerly a car driver, now an inmate of City Home. As a young man he served in the army at the outbreak of the Civil War. He says that he has never been sick, except two years ago, when he was in the hospital for two months with fever. The hair in the axillae has always been scant and he has never had any hair on his thorax. During most of his life he drank about a pint of whisky a day but has had none for six months. He has had no libido for seven or eight years. Recently he has been spending his winters in the City Home and his summers at work. He feels perfectly well and gives no symptoms except frequency of urination at night.

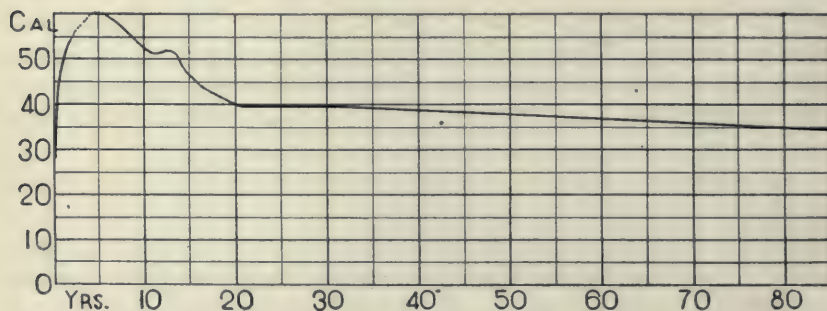


Fig. 1.—Curve showing the level of metabolism at different ages. Results are expressed in terms of calories per hour per square meter of surface area (Linear and Height-Weight Formulas). In accordance with the findings in the present series, the line is somewhat higher in old age than in the curves published in previous papers.

**Physical Examination.**—A well nourished man, with white hair and slightly wrinkled skin, who looks about 65 years old. No arcus senilis. Heart sounds are of good quality, with an occasional premature systole. There is a faint blowing systolic murmur at the apex. Blood pressure: 235 mm. systolic; 100 mm. diastolic. The pubic hair is scant and of feminine type. Genitalia normal.

The man was of active disposition and spent most of his time walking about the ward. He was the least senile of the group.

**CASE 2.—History.**—Henry L., 78 years old, formerly a baker, now an inmate of City Home. Six years ago he broke his leg and was in the hospital five months. He passes urine twice at night. He has used alcohol sparingly, but has taken a great deal of snuff.

**Physical Examination.**—Well nourished, skin wrinkled, looks his full age. There is a slight tremor of the hands. Thorax deep, breath sounds emphysematous, with fine râles at the bases. The left border of the heart percusses



13 cm from the median line. There are a few premature systoles; no murmurs. The radial walls are not palpable. Blood pressure: 155 mm. systolic; 80 mm. diastolic.

His disposition was quiet and he spent most of his time in the hospital sitting in a chair. The first time he was in the calorimeter, March 13, he did not understand the instructions and was very restless, taking snuff, coughing, sneezing and moving about. The second time he was in the calorimeter, March 18, he was quiet and the observation was satisfactory.

**CASE 3.—History.**—Charles H., 79 years old, formerly a cigar maker, now in City Home. Ten brothers and sisters lived to be over 70 years of age. Two years ago he had pneumonia. He passes urine two or three times at night. He has been a moderate drinker of beer and a heavy smoker. No libido for the past five years. He feels perfectly well except for a slight cough in the morning.

**Physical Examination.**—Rather thin, skin flabby and much wrinkled; well preserved for his age. Thorax deep; breath sounds emphysematous. Left border of cardiac dullness 15 cm. from median line. Blood pressure: 210 mm. systolic; 100 mm. diastolic. Urine contains trace of albumin.

His disposition was active and restless and he spent most of his time standing about the ward or working.

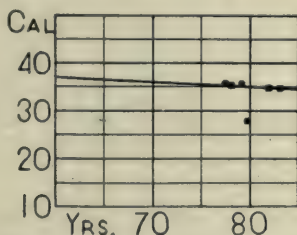


Fig. 2.—Part of Figure 1 with dots representing the findings in the old men of this series.

**CASE 4.—History.**—Charles W., 80 years old, formerly a waiter, for the last year and a half an inmate of City Home. Two years ago his arm was injured in an accident. He was formerly very alcoholic. Fifty-five years ago he had lues. No libido for seven years.

**Physical Examination.**—Well nourished, skin wrinkled, left border of cardiac dullness 14 cm. to left of median line. At the apex there is a faint systolic murmur. Blood pressure: 180 mm. systolic; 80 mm. diastolic. Breath sounds are emphysematous, with coarse râles at the bases. The urine is normal. Wassermann reaction negative.

This was the most senile of the group. All day long he sat motionless in his chair, taking but little interest in his surroundings.

**CASE 5.—History.**—William C., 83 years old, formerly a newspaper writer, now waiting to be admitted to the City Home. He has had no alcohol for the last forty years, but has smoked excessively. His health has always been good. One week ago he developed a slight cough.

**Physical Examination.**—Well nourished, skin wrinkled, hair white. Heart normal except for occasional premature systoles. The breath sounds are emphysematous. His disposition is cheerful and he is fairly active. The urine contained 49 gm. glucose on a carbohydrate intake of 190 gm. daily. He had no acidosis.

**CASE 6.—History.**—John B., 83 years old, formerly a storekeeper, now waiting in Bellevue Hospital until he can find a place in a home for the aged.

TABLE 1.—OLD MEN. DATA OF—

Subject, Date, Weight, Surface Area, Linear Formula	Period	End of Period	Carbon Dioxid, Gm.	Oxygen, Gm.	R. Q.	Water, Gm.	Urine N per Hour, Gm.	Indirect Calo- rimetry, Cal.	Heat Elimi- nated, Cal.
Case 1..... (Andrew O'C.) 5/8/16 69.7 Kg. 1.87 Sq. M.	Prelim.	10:35	.....	.....	.....	.....	.....	.....	.....
	1	11:35	22.3	20.0	0.82	34.6	0.562	66.3	78.0
	2	12:35	22.5	20.0	0.82	34.6	0.562	66.3	78.0
	Aver.	.....	.....	.....	.....	.....	.....	66.6	.....
Case 2 (Henry L.) 3/13/16 67.9 Kg. 1.85 Sq. M.	Prelim.	11:43	.....	.....	.....	.....	.....	.....	.....
	1	12:43	24.6	22.2	0.81	31.6	0.2885	74.2	75.3
	2	1:43	25.1	24.2	0.76	34.6	0.2885	79.8	79.9
	Aver.	.....	.....	.....	.....	.....	.....	.....	.....
Henry L. .... 3/13/16 68.9 Kg. 1.85 Sq. M.	Prelim.	11:31	.....	.....	.....	.....	.....	.....	.....
	1	12:31	22.0	18.0	0.89	19.9	0.291	61.3	63.9
	2	1:31	23.8	20.7	0.84	25.0	0.291	69.5	72.7
	Aver.	.....	.....	.....	.....	.....	.....	.....	.....
Case 3 (Chas. H.) 5/3/16 52.9 Kg. 1.66 Sq. M.	.....	10:26	.....	.....	.....	.....	.....	.....	.....
	1	11:16	15.7	14.1	0.81	22.4	0.238	47.2	46.0
	2	12:26	23.4	21.2	0.80	32.1	0.238	70.8	70.9
	Aver.	.....	.....	.....	.....	.....	.....	.....	.....
Case 4 (Chas. W.) 5/4/16 69.1 Kg. 1.82 Sq. M.	Prelim.	10:31	.....	.....	.....	.....	.....	.....	.....
	1	11:31	17.3	15.4	0.82	27.5	0.228	51.5	60.4
	2	12:31	16.9	15.0	0.82	25.5	0.228	50.2	59.8
	Aver.	.....	.....	.....	.....	.....	.....	.....	.....
Case 5 (Wm. C.) 3/2/16 62.9 Kg. 1.83 Sq. M.	Prelim.	11:39	.....	.....	.....	.....	.....	.....	.....
	1	12:39	20.0	17.0	0.86	22.6	Lost	57.3*	57.4
	2	1:39	21.7	18.1	0.87	24.4	Lost	61.5*	65.7
	Aver.	.....	.....	.....	.....	.....	.....	.....	.....
Case 6 (John B.) 11/4/15 50.5 Kg. 1.48 Sq. M.	Prelim.	11:55	.....	.....	.....	.....	.....	.....	.....
	1	12:55	16.7	15.5	0.78	21.7	0.268†	51.2	51.4
	2	1:55	16.4	15.8	0.76	21.8	0.268†	52.1	54.3
	Aver.	.....	.....	.....	.....	.....	.....	.....	.....

\* Calculated from table of Magnus-Levy, assuming 15 per cent. of calories from protein.

† Calculated from 24-hour specimen.

877

—CALORIMETER EXPERIMENTS

Direct Calorimetry, Cal.	Rectal Temp., C.	Average Pulse	Work-Adder, Cm.	Non-protein R. Q.	Per Cent. Calories from			Calories per Hour		Remarks
					Protein	Fat	Carbo-hyd.	Per Kg.	Per Sq. M. (Lin.)	
.....	37.2	....	.....	.....	..	..	..	....	....	Basal; in bed
69.0	37.1	61	14	0.80	..	..	..	....	....	Very quiet
74.4	36.9	59	11	0.82	..	..	..	....	....	Very quiet
.....	.....	.....	.....	.....	22	50	28	0.95	35.6	
.....	37.2	69	.....	.....	..	..	..	....	....	In bed; 18 hours after food
70.1	37.1	69	34	0.81	..	..	..	....	....	Restless, coughing
80.4	37.2	66	34	0.75	..	..	..	....	....	Restless, coughing (observation not included in averages)
.....	.....	.....	.....	.....	10	68	22	1.13	41.6	Basal; in bed
.....	37.1	74	.....	.....	..	..	..	....	....	
56.7	36.9	70	7	0.90	..	..	..	....	....	Very quiet
69.4	36.9	70	30	0.84	..	..	..	....	....	Quiet
.....	.....	.....	.....	.....	12	39	49	0.95	35.5	
.....	37.4	.....	.....	.....	..	..	..	....	....	Basal; in bed
42.7	37.3	72	16	0.81	..	..	..	....	....	Very quiet; 50 min. period
72.0	37.4	74	47	0.80	..	..	..	....	....	Fairly quiet; 70 min. period
.....	.....	.....	.....	.....	11	60	29	1.12	35.6	
.....	37.0	.....	.....	.....	..	..	..	....	....	Basal; in bed
49.2	36.8	55	4	0.82	12	54	34	....	....	Motionless
56.1	36.7	55	7	0.82	12	65	23	....	....	Almost motionless
.....	.....	.....	.....	.....	..	..	..	0.74	27.9	
.....	36.7	.....	.....	.....	..	..	..	....	....	
55.1	36.7	66	6	.....	..	..	..	....	....	Very quiet; dozed 45 minutes
66.0	36.7	63	18	.....	..	..	..	....	....	Quiet; awake
.....	.....	.....	.....	.....	..	..	..	0.94	34.3	
.....	36.8	.....	.....	.....	..	..	..	....	....	Basal; in bed
45.7	36.7	49	28—	0.78	14	64	22	....	....	Fairly quiet
52.5	36.7	51	33—	0.75	14	74	12	....	....	Restless last 10 minutes
.....	.....	.....	.....	.....	..	..	..	1.02	34.9	



His mother died at the age of 93. The patient himself has always been rather weak physically. Over twenty-five years ago he had malaria and yellow fever, which caused deafness in the right ear. Ten years ago he discovered that his right eye was almost blind. For the last two or three years his memory has been failing, particularly for recent events. He has been a heavy smoker and when young was moderately alcoholic. At present he has marked nocturia. He kept at his clerical work until July, 1915.

*Physical Examination.*—Well preserved old man, skin only slightly wrinkled, moderate flabbiness of flesh, slight tremor of hands, gait shuffling. The right eye shows a cataract. The heart is slightly enlarged, with an occasional premature systole. There is a faint systolic murmur at the apex and a rough diastolic murmur. Radials are faintly palpable. The lungs are emphysematous. Blood pressure: systolic, 180 mm.; diastolic, 100 mm. Temperature normal; pulse, 55 to 75.

This patient was becoming senile rather rapidly. At the time of the calorimeter observation he was fairly active and spent some of his time walking about the ward. A month or so later he was almost blind, failing mentally and glad to stay in his chair all day.

#### DISCUSSION OF RESULTS

The calorimeter findings on the group of old men can be compared directly with the findings on Boy Scouts and normal adults between the ages of 20 and 47. The experimental methods used with all of these subjects were the same in almost all particulars. There was, however, one difference between the groups which could not be avoided. The younger subjects were active men on liberal diets, and almost all of them came to the calorimeter room from their homes on the mornings of the experiments. The old men were beyond the working age, they had been on rather meager fare, and they were all kept in the metabolism ward for several days before the tests were made. All of these factors tend to decrease the total metabolism in the same manner in which cage life affects a dog.<sup>2</sup> Still they are part and parcel of old age. It would be difficult to find hard working men of 80 on large diets, or normal men in the prime of life leading the existence of octogenarians.

If we look at the results of the calorimeter experiments we are struck by their uniformity in five of the observations. The first experiment on Henry L. is published for the sake of completeness, but it cannot be considered a test of the basal metabolism. The old man was stupid and did not understand the instructions to stay quiet. He turned frequently from side to side and inhaled snuff which he had smuggled into the calorimeter. This made him cough and sneeze. The second time he was in the calorimeter he was quiet and the observation was very satisfactory. It will be noted that the metabolism of Charles W. was unusually low. This may be accounted for by the fact that he was much more senile than the others. While this finding is of

2. Lusk, G.: Animal Calorimetry, Paper 11, Jour. Biol. Chem., 1915, **20**, 565; and Allen and Du Bois: Clinical Calorimetry, Paper 17, THE ARCHIVES INT. MED., 1916, **17**, 1056.

TABLE 2.—OLD MEN. CLINICAL DATA<sup>1</sup>

Case Number Name and Date	Food			Food N., Gm.	Urine N., Gm.	Feces N., Gm.	Ex- creta N., <sup>2</sup> Gm.	Nitro- gen Bal- ance, Gm.	Body Wt., Kg.	Urine Vol- ume, C.c.	Calo- ries per Kg.	Blood Pres- sure
	Total Calo- ries	Car- bo- hyd., Gm.	Fat, Gm.									
Case 1 Andrew O'C. 5/ 7/16	2,354	223	125	10.7	9.1	1.1	10.1	+0.6	....	1,100		
5/ 8/16	2,524	240	135	11.2	9.5	1.1	10.6	+0.6	....	1,675		
5/ 9/16	2,592	278	136	7.2	9.4	0.7	10.1	-2.9	70.2	1,420	37	
5/10/16	2,443	277	130	4.0	7.2	0.4	7.6	-3.6	....	1,760	..	235-100
5/11/16	3,043	445	120	4.0	7.2	0.4	7.6	-3.6	70.2	1,630	29	
5/12/16	3,043	436	125	4.1	4.0	0.4	4.4	-0.4	70.1	1,440	29	
Case 2 Henry L. 3/10/16	2,766	286	129	15.4	8.8 <sup>3</sup>	1.5	10.3	+5.1	....	1,600 <sup>3</sup>		
3/11/16	2,885	310	135	13.9	8.2	1.4	9.5	+4.4	69.1	760	42	
3/12/16	2,824	304	129	15.0	9.0 <sup>4</sup>	1.5	10.5	+4.5	69.1	1,030 <sup>4</sup>	41	
3/13/16	.....	...	...	....	6.1 <sup>5</sup>	...	....	.....	67.9	1,037 <sup>5</sup>	..	154-80
3/14/16	2,924	323	131	14.7	7.2	1.5	8.7	+6.0	68.7	580	41	
3/15/16	3,511	353	180	14.3	7.5	1.4	8.9	+5.4	69.1	650	50	
3/16/16	3,577	352	186	15.6	10.4	1.6	11.9	+3.7	69.6	960	51	
3/17/16	3,460	326	186	15.4	7.1	1.5	8.6	+6.8	69.5	600	50	
3/18/16	.....	...	...	....	...	...	....	.....	....	.....	..	153-100
Case 3 Charles H. 5/3/16	1,954	184	101	10.2	7.4	1.0	8.4	+1.8	52.9	1,045	..	210-100
5/4/16	2,299	218	121	11.0	8.2	1.1	9.3	+1.7	53.6	800	43	
Case 4 Charles W. 5/3/16	2,008	220	92	9.8	6.4	1.0	7.4	+2.4	....	1,480		
5/4/16	1,765	153	97	9.1	7.0	0.9	7.9	+1.2	69.1	1,660	..	180-100
Case 5 William C. 3/2/16	1,825 <sup>7</sup>	191 <sup>6</sup>	82	11.0	11.3	...	....	.....	62.9	1,940 <sup>6</sup>	29	
Case 6 John B. 11/ 4/15	1,364	126	69	7.9	6.6	...	....	.....	50.5	1,230	27	Syst. 180-190 Diast. 100
12/12/15	1,450	195	60	3.4								
12/13/16	1,326	189	51	3.2	4.1	...	....	.....	51.0	700		

1. Temperature normal in all cases throughout the experiments.

2. Excreta nitrogen calculated as urine nitrogen plus 10 per cent. of food nitrogen.

3. Twenty-three hours fifteen minutes.

4. Twenty-three hours thirty minutes.

5. Twenty-three hours thirty minutes.

6. 48.7 gm. glucose in urine.

7. Approximately.

importance in showing the great depression in metabolism which may occur in old age, we are not justified in using it to obtain the average figure which represents the heat production of men of his age. The average for the other five men was 35.1 calories per square meter per hour, and the average deviation from this mean was a little over 1 per cent. When we take the group of fifteen younger men studied in the calorimeter, we find an average deviation from the mean of about 3 per cent. The results on Charles W. show a deviation of 21 per cent. from the average of the other old men. He is therefore excluded from the averages as the result of the rule which debar an observation in which the deviation from the mean is greater than four times the average deviation.

TABLE 3.—OLD MEN. SUMMARY

Case Number and Name	Age, Yrs.	Weight, Kg.	Height, Cm.	Signs of Senility	Surface Area (Lin.) Sq. M.	Total Calories in 2 Hours		Cal. per Hour		Per Cent. from Adult, Av. 39.7	Av. R. Q.	Av. Pulse
						Indirect	Direct	Per Kg.	Per Sq. M.			
1. Andrew O'C.	77	69.7	171	+	1.87	133.3	143.4	0.962	35.6	-10	0.81	59
2. Henry L. ....	78	67.9	167	++	1.85	154.0	150.5	1.13	(41.6)*	(+ 5)*	0.78	68
Henry L. ....	..	68.9	...	....	1.85	130.7	126.1	0.949	35.3	-11	0.86	71
3. Charles H. ...	79	52.9	163	+	1.66	118.0	114.6	1.12	35.6	-10	0.80	73
4. Charles W. ...	80	69.1	164	+++	1.82	101.7	105.3	0.736	27.9†	-30†	0.82	55
5. William C. ...	83	62.9	163	++	1.73	118.8	121.2	0.944	34.3	-14	0.86	65
6. John B. ....	83	50.5	158	+++	1.48	103.3	98.2	1.02	34.9	-12	0.77	50
Total or aver.	..	....	...	....	.....	859.3	859.3	.....	35.1	....	0.81	

\* Excluded from averages on account of restlessness.

† Excluded from averages on account of unusually low metabolism.

The average heat production of the five men between the ages of 77 and 83 was 35.1 calories per square meter per hour, which is about 12 per cent. below the average for men between the ages of 20 and 50. This depression in old age is about 7 per cent. less than had been assumed by one of us in a curve based on results obtained by other investigators.<sup>3</sup> As one might expect, the depression of metabolism is somewhat proportional to the degree of senility.

The respiratory quotients lie between the figures 77 and 86, the average being 81. The method of direct calorimetry gives a total of 859.3 calories for the seven experiments, which is within 0.1 per cent. of the total obtained by the independent method of indirect calorimetry (859.8 calories). This agreement is unusually close for two-hour experiments.

3. Du Bois, E. F.: The Respiration Calorimeter in Clinical Medicine, Am. Jour. Med. Sc., 1916, **151**, 781.



## SUMMARY AND CONCLUSIONS

A group of six men between the ages of 77 and 83 were studied in the calorimeter. Their average basal heat production was 35.1 calories per square meter per hour, which is 12 per cent. below the average for men between the ages of 20 and 50. The methods of direct and indirect calorimetry agreed closely and the respiratory quotients were all within normal limits.

NOTE.—At the suggestion of Drs. Means and Boothby, a set of normal standards for various ages has been calculated from the age curve published in this paper and Lusk's "Science of Nutrition," third edition, 1917. These will be used in the Sage publications after the present series, until changes in the curve are made by the addition of new data. The figures for females are calculated as 7 per cent. below the average for males, more data, however, being desirable to establish for the female sex the general validity of this method of computation.

CALORIES PER SQUARE METER OF BODY SURFACE PER HOUR  
(HEIGHT-WEIGHT FORMULA)

Age, Years	Males	Females
14-16.....	46.0	43.0
16-18.....	43.0	40.0
18-20.....	41.0	38.0
20-30.....	39.5	37.0
30-40.....	39.5	36.5
40-50.....	38.5	36.0
50-60.....	37.5	35.0
60-70.....	36.5	34.0
70-80.....	35.5	33.0

477 First Avenue.

# CLINICAL CALORIMETRY

TWENTIETH PAPER

## THE EFFECT OF CAFFEIN ON THE HEAT PRODUCTION\*

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AND

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WITH THE TECHNICAL ASSISTANCE OF G. F. SODERSTROM

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The object of this research was to determine the effect of a single large dose of caffein on the respiratory metabolism of normal individuals.

The earliest work on the effect of this drug on the gaseous exchange, so far as we have been able to discover, was that of Hoppe<sup>1</sup> in 1857. He found some increase in the carbon dioxid output after taking caffein, but the conditions of his experiments are open to criticism. He was followed two years later by Smith<sup>2</sup> who studied in a masterly way nearly every aspect of respiration, including the effect of tea, coffee, etc. His technic, so far as one can judge from the description, was excellent. His apparatus was of the open circuit type, the subject breathing through a mask and valves. The inspired air went first through a dry meter and the expired air first through a Woulff bottle, containing pumice stone saturated with sulphuric acid, then through a gutta-percha box having a series of compartments in which the air was exposed, over a large surface, to concentrated potassium hydrate. Next, it was passed through a second Woulff bottle with sulphuric acid, the carbon dioxid was determined by the gain in weight of the absorbers, exactly as is done with the modern calorimeter. The total ventilation of the lungs, the evaporation of moisture from the lungs, the respiration rate and pulse rate were the other factors studied. The oxygen, of course, was not obtained. After taking from 50 to 100 grains of black or green tea leaves, or half an ounce of strong coffee, a rise in the carbon dioxid output was invariably found with

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\* Submitted for publication Dec. 19, 1916.

† From the Russell Sage Institute of Pathology, in affiliation with the Second Medical Division, Bellevue Hospital.

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1. Hoppe: Ueber die Wirkungen des Coffein, Deutsch. Klin., 1857, **9**, 181.

2. Smith, Edward: Experimental Inquiries Into the Chemical and Other Phenomena of Respiration and Their Modifications by Various Physical Agencies, Philosophical Transactions, London, 1859, **149**, 681.

several normal subjects. A very large number of experiments were performed. The rise was anywhere from 15 to 30 per cent. and the maximum usually occurred within an hour from the time of taking the drug. An increase in the ventilation of the lungs of similar proportion was also found. The respiratory rate was sometimes increased, sometimes decreased. The pulse was usually slightly accelerated.

The next extensive study of the effect of caffein on the metabolism, so far as we are aware, is that of Reichert<sup>3</sup> in 1890. This observer worked with dogs, his apparatus being a simple type of water calorimeter. The method of direct calorimetry alone was used. His work may be subject to some criticism, for the dogs were tied down and occasionally struggled. The results of his normal control experiments sometimes differed rather widely, but the faults were remedied to some extent by the very large number of experiments made. The caffein was given subcutaneously. A basal hour was secured first and the dogs were then observed for five hours after receiving the drug. Three series of experiments were performed. In the first series the dose was 0.035 gm. per kg. Five experiments were made. The average increase in heat production was 38.6 per cent. Heat elimination was also increased, but in relation to production was inconstant. In three experiments heat elimination was in excess; in the other two it was not. In the second series, 0.07 gm. per kg. were given. The average increase in heat production was 43.6 per cent. The maximum was reached in two experiments during the first hour after administering the drug; in three, during the second hour. In all the experiments of this series, heat production was in excess of elimination during the first hour, and in three, in excess during the subsequent hour. More heat was eliminated than produced during the remaining hours. In the third series, 0.105 gm. per kg. was given. (The lethal dose was from 0.15 to 0.20 gm. per kg.). In this series there was an average increase in heat production of 71.4 per cent. In all the experiments heat production was in excess of elimination in the first hour and in three also in the second hour. In summing up, Reichert says, "These results show beyond possible doubt that caffein increases heat production, and as a corollary, increases destructive tissue metamorphosis."

More recently, Edsall and Means<sup>4</sup> reported experiments on the effect of caffein on two normal men. In one, after 0.324 gm. of caffein

3. Reichert, E. T.: Action of Caffein on Tissue Metamorphosis and Heat Phenomena, *New York Med. Jour.*, 1890, **51**, 456. For a description of his apparatus see *Heat Phenomena in Normal Animals*, *University Med. Mag.*, Philadelphia, 1890, **2**, 173, 225, 345.

4. Edsall, D. L., and Means, J. H.: Effect of Strychnin, Caffein, Atropin and Camphor on the Respiration and Respiratory Metabolism in Normal Human Subjects, *THE ARCHIVES INT. MED.*, 1914, **14**, 897.



Subject, Date, Weight, Surface Area, Linear Formula	Period	End of Period	Carbon Dioxid, Gm.	Oxygen, Gm.	R. Q.	Water, Gm.	Urine N per Hour, Gm.	Indirect Calo- rimetry, Cal.	Heat Elimi- nated, Cal.
Case 1..... (E. F. D. B.) 4/12/16 76.5 Kg. 1.94 Sq. M.	Prelim.	11:32	.....	.....	.....	.....	.....	.....	.....
	1	12:32	25.4	22.7	0.81	31.5	0.502	75.5	82.4
	2	1:32	24.7	22.7	0.79	32.7	0.502	75.3	82.0
Av. basal.....	.....	.....	.....	.....	.....	.....	.....	75.4	.....
	3	2:32	27.8	25.7	0.79	37.8	0.688	84.7	87.4
	4	3:32	25.6	23.5*	0.79	37.1	0.688	77.5	82.4
Av. after caffein	.....	.....	.....	.....	.....	.....	.....	.....	.....
E. F. D. B.....	Prelim.	11:07	.....	.....	.....	.....	.....	.....	.....
	1	12:07	24.4	22.1	0.80	33.3	0.536	73.2	78.9
	2	1:07	27.1	Lost	.....	34.7	0.705	83.5†	82.0
	3	2:07	25.9	Lost	.....	35.7	0.705	78.9†	83.3
	4	3:07	25.8	24.6	0.77	33.8	0.579	80.7	79.5
	5	4:07	26.2	23.7	0.81	34.9	0.579	78.6	81.6
Av. after caffein	.....	.....	.....	.....	.....	.....	.....	80.6	.....
Case 2 (J. H. M.)..	Prelim.	11:26	.....	.....	.....	.....	.....	.....	.....
	1	12:26	24.2	21.9	0.80	32.3	0.524	72.8	81.3
	2	1:26	25.1	23.1	0.79	34.9	0.524	76.4	87.5
Av. basal.....	.....	.....	.....	.....	.....	.....	.....	74.6	.....
	3	2:26	29.5	26.5	0.81	36.1	0.604	88.1	84.3
	4	3:26	28.9	27.3	0.77	41.2	0.604	89.9	90.0
	5	4:26	31.5	29.0	0.77	42.2	0.618	98.4	89.7
Av. after caffein	.....	.....	.....	.....	.....	.....	.....	92.1	.....
Case 3 (J. C. F.)...	Prelim.	11:39	.....	.....	.....	.....	.....	.....	.....
	1	12:39	23.6	21.2	0.81	26.8	0.592	70.4	70.5
	2	1:39	25.9	22.7	0.83	28.6	0.592	75.7	77.4
Av. basal.....	.....	.....	.....	.....	.....	.....	.....	73.1	.....
	3‡	2:42	26.3	24.7	0.78	36.9	0.631	81.1	87.0
	4§	3:39	25.8	22.8	0.82	41.7	0.516	76.1	81.8
	5	4:39	26.6	23.3	0.83	34.3	0.414	78.0	76.6
Av. after caffein	.....	.....	.....	.....	.....	.....	.....	78.4	.....
Case 4 (J. C. A.)...	Prelim.	11:10	.....	.....	.....	.....	.....	.....	.....
	1	12:10	22.3	19.1	0.85	29.6	0.546	64.0	70.3
	2	1:10	22.8	19.2	0.87	29.6	0.546	64.5	72.4
Av. basal.....	.....	.....	.....	.....	.....	.....	.....	64.3	.....
	3	2:10	25.4	21.9	0.84	32.6	0.546	73.4	75.2
	4	3:10	26.4	24.6	0.78	38.4	0.546	81.2	81.6
	5	4:10	22.6	20.2	0.81	33.4	0.546	66.9	73.1
Av. after caffein	.....	.....	.....	.....	.....	.....	.....	73.8	.....

\* Small leak found in blower this hour.

† Calculated from CO<sub>2</sub>, R. Q. assumed to be 0.79.

‡ 63-minute period.

§ 57-minute period.

## —CALORIMETER EXPERIMENTS

Direct Calorimetry Calories	Rectal Temp., C.	Average Pulse	Work-Adder, Cm.	Non-protein R. Q.	Per Cent. Calories from			Calories per Hour		Remarks
					Protein	Fat	Carbohyd.	Per Kg.	Per Sq. M.	
.....	36.9	62								
80.8	36.8	56	9	0.82	..	..	..	....	38.9	Basal; very quiet; on right side
81.0	36.8	58	24	0.79	..	..	..	....	38.8	Basal; very quiet; on back
.....	.....	.....	.....	.....	18	56	26	0.99	38.8	Caffein, 0.65 gm. at 1:33 p. m.
89.1	36.9	56	22	0.78	22	58	20	....	43.6	Very quiet
81.5	36.9	55	14	0.79	24	55	21	....	40.0	Very quiet
.....	.....	.....	.....	.....	..	..	..	....	41.8	
.....	36.9									
71.5	36.8	56	28	0.80	19	54	27	0.95	37.5	Basal; quiet
78.5	36.8	55	30	.....	..	..	..	....	42.8	Caffein, 0.65 gm. at 12:08 p. m.; quiet
86.3	36.9	60	13	.....	..	..	..	....	41.0	Very quiet; on right side
79.8	36.9	55	11	0.76	19	67	14	....	41.4	Very quiet; on back
82.4	37.0	56	21	0.81	20	53	27	....	40.3	Quiet
.....	.....	.....	.....	.....	..	..	..	1.04	41.4	
.....	36.9									
71.6	36.7	59	26	0.80	..	..	..	....	38.5	Basal; fairly quiet
83.4	36.7	55	30	0.79	..	..	..	....	40.4	Basal; fairly quiet
.....	.....	.....	.....	.....	19	57	24	0.99	39.5	Caffein, 0.65 gm. at 1:27 p. m.
90.4	36.8	57	21	0.81	18	53	29	....	46.6	Fairly quiet
92.6	36.9	62	32	0.76	18	67	15	....	47.5	Fairly quiet
96.5	37.0	77	39	0.76	17	68	15	....	52.0	Fairly quiet
.....	.....	.....	.....	.....	18	63	19	....	48.8	
.....	36.3									
71.2	36.3	....	19	0.81	22	50	28	....	40.2	Basal; very quiet
78.0	36.4	....	45	0.84	21	43	36	....	43.3	Fairly quiet; reading
.....	.....	.....	.....	.....	22	46	32	1.14	41.7	Caffein, 0.54 gm. at 1:43 p. m.
81.5	36.3	....	33	0.77	22	62	16	....	46.4†	Very quiet
73.8	36.2	....	33	0.83	17	49	34	....	43.5‡	Very quiet; slept 25 minutes
77.0	36.3	....	32	0.84	14	48	38	....	44.5	Very quiet
.....	.....	.....	.....	.....	18	53	29	....	44.8	
.....	36.8									
75.9	36.9	66	18	0.86	..	..	..	....	39.0	Basal; very quiet
73.6	36.7	65	9	0.89	..	..	..	....	39.3	Basal; very quiet
.....	.....	.....	.....	.....	..	..	..	1.07	39.1	Caffein, 0.525 gm. at 1:11 p. m.
74.0	36.9	61	21	0.85	..	..	..	....	....	Fairly quiet
80.6	36.9	59	46	0.77	..	..	..	....	49.5	Fairly quiet
67.7	36.8	59	18	0.82	..	..	..	....	40.7	Very quiet
.....	.....	.....	.....	.....	23‡	33‡	44‡	1.23	45.0	

† Average of whole experiment.

sodium salicylate, given subcutaneously, there was an average rise of 10.6 per cent. in the metabolism, as measured by indirect calorimetry. The maximum of 13.9 per cent. was reached in the first hour. With the other subject there was an average rise of 3 per cent. and a maximum of 5.7 per cent. after 0.39 gm., given in the same way.

Higgins and Means<sup>5</sup> obtained similar results. In one normal subject, after 0.32 gm. of caffein sodium benzoate subcutaneously there was an average rise of 13.8 per cent. in the indirect calorimetry. The maximum rise of 15.4 per cent. occurred within the first hour. In another normal subject, 0.32 gm. of the same drug was given subcutaneously and was followed by an average rise of 4.5 per cent. and a maximum of 7.2 per cent., which occurred during the second hour.

In neither Edsall and Means' nor in Higgins and Means' experiments was there any consistent change in the respiratory quotient after the drug; in other words, the proportion of fat, carbohydrate and protein utilized showed no regular deviation from the normal.

#### METHODS OF EXPERIMENTS

Five calorimeter experiments on four normal men were carried out in precisely the same way as already described in the preceding papers of this series.<sup>6</sup> The surface area of all the subjects was determined by the so-called "Linear Formula," described in Papers 5, 9 and 10. The calories derived from protein were calculated from specimens voided between the hours of 8 a. m. and 5 p. m.

The caffein was taken in the form of the pure alkaloid dissolved in water. A tumbler containing the caffein dissolved in 300 c.c. of water at room temperature was placed in the calorimeter when the subject entered. After a preliminary period and one or two hours of observation of the subject's normal basal metabolism, he was told to drink the mixture and was allowed to follow it with a little pure water. After the taking of the drug the observation was continued for three hours, except in one experiment, when the third hour was omitted.

The subjects were studied in the morning hours at complete rest, fourteen to eighteen hours after the last meal. They were not allowed to take coffee or tea for the twenty-four hours previous to the test.

The data of all the experiments are given in the accompanying table.

#### DESCRIPTION OF SUBJECTS OF EXPERIMENTS

CASE 1.—E. F. D. B., man, aged 34, height 178 cm., physician. For history see Fourth Paper of this series. Since last year he has been in good health; has taken very little exercise since September, 1915. Takes one medium-sized

5. Higgins, H. L., and Means, J. H.: Effect of Certain Drugs on the Respiration and Gaseous Metabolism of Normal Human Subjects, *Jour. Pharmacol. and Exper. Therap.*, 1915, **7**, 1.

6. Papers 1 to 8: *THE ARCHIVES INT. MED.*, 1915, **15**, 793-944; Papers 9 to 17: *Ibid.*, 1916, Part 2, **17**, 855-1059.



cup of coffee at breakfast and occasionally tea in the afternoon, or a small cup of coffee after dinner. Is kept awake by coffee or tea taken after 1 p. m. Tobacco, moderate. Alcohol, occasionally in small amounts.

CASE 2.—J. H. M., man, aged 31, height 177 cm., physician. Measles, mumps, scarlet fever and chickenpox in childhood; a bronchopneumonia at 26 and paratyphoid at 28; otherwise, has always enjoyed good health. Takes exercise only spasmodically, but leads a fairly active life.

Takes one cup of coffee in the morning about half the time, a small cup of coffee after dinner about twice a week, one or two cups of tea in the afternoon about four times a week. One demitasse will not keep him awake; a large cup of coffee in the evening will. Tobacco, moderate. Alcohol, occasionally in small amounts.

CASE 3.—J. C. F., man, aged 23, height 178 cm., chemist. Pleurisy and pneumonia at 6; scarlet fever at 13; occasional sore throat. In good health except for slight nasal obstruction. Exercise: walks three to four miles a day.

He takes a cup of coffee at each meal and sometimes a cup late at night without being kept awake. Tobacco, three to five cigars a day. Alcohol, none.

CASE 4.—J. C. A., man, aged 26, height 175.2 cm., physician. Scarlet fever nine years ago; catarrhal jaundice five years ago; in good health since. Leads a sedentary life, but always takes some exercise.

Takes two cups of coffee a day; no tea. Even large quantities of coffee in the evening never keep him awake. Tobacco, ten cigarets a day. Alcohol, occasionally in small amounts.

#### DISCUSSION OF RESULTS

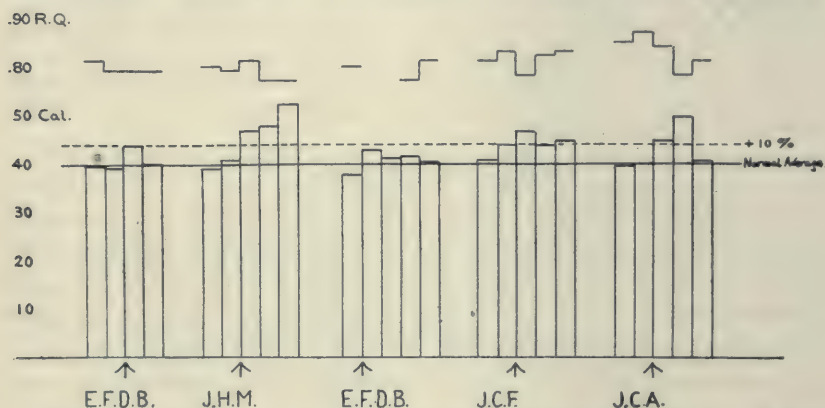
*The Basal Metabolism After Giving Caffein.*—This was increased in each experiment. The dose given, 8 to 10 grains, approximately 8.6 mg. per kg. of body weight, is considerably larger than the usual therapeutic dose. In all five experiments there was a rise in the basal metabolism (indirect calorimetry). This rise varied from the minimum of 11.3 per cent. with J. C. F., to the maximum of 31.6 per cent. with J. H. M. The highest average rise was that of J. H. M., 23.5 per cent., and the lowest that of J. C. F., of 7.4 per cent. In three experiments, both those on E. F. D. B. and that on J. C. F., the maximum rise occurred in the first hour after receiving the drug. In the experiment on J. C. A., the maximum rise occurred in the second hour, and that in J. H. M. in the third hour. In none of these experiments had the metabolism returned to the basal value when the observation was discontinued. The increments in the metabolism obtained in this series of experiments are definitely greater than those obtained by Edsall and Means and by Higgins and Means when using much smaller doses of the drug. The rises in the basal metabolism of the present experiments are shown graphically in the accompanying figure.

As one looks over the enormous literature on the various actions of caffein, one gets the impression that the numerous contradictory statements are not necessarily due entirely to faulty observation, but possibly because the action of caffein on any given function is extremely variable, often diametrically opposite, in different individuals, and in the same individual with varying dosage. Nevertheless, the present

series of experiments, together with those in the literature, show that an elevation in the basal metabolism is a very frequent action, although its intensity varies considerably in different individuals. In general, there seems to be a larger rise with larger doses.

*The Respiratory Quotients.*—These show no consistent change in either direction after the drug had been taken.

*The Methods of Direct and Indirect Calorimetry.*—These agree closely in the hours following the taking of caffeine. The total calories as measured by the former method in the hours following the drug were 1,230.3. This is within 1 per cent. of the total of 1,218.0 calories as measured by the method of indirect calorimetry. This agreement of two absolutely independent methods is unusually close, and it confirms the technical accuracy of the findings. The drug had little effect on



Results shown graphically. Columns represent calories per hour as measured by indirect calorimetry. Arrows indicate point at which the caffeine was taken. The topmost lines show the respiratory quotients.

the body temperature and the differences between heat elimination and heat production are such as would be found in experiments on normal controls.

The agreement between the methods of direct and indirect calorimetry in the basal hours before the drug was taken is not very close. The direct method gives a total of 687.6 calories; the indirect, 647.8 calories. The method of direct calorimetry is seldom very satisfactory in the first hour of an observation, and these one- or two-hour basal experiments are not long enough to balance up the inequalities. With longer experiments on normal controls the agreement is within a fraction of 1 per cent., as has been shown in previous papers.

*The Elimination of Water from Skin and Lungs.*—This was increased, after taking the caffeine, in every experiment. This was greatest in the case of J. C. F., being 36 per cent. and least in the second

experiment on E. F. D. B., when it was but 5 per cent. The average percentage of calories lost in the vaporization of water during the basal hours was 24.1 per cent.; after taking the caffein the average was 26.3 per cent. The drug affects but slightly the mechanism by which the body dissipates heat in response to an increased heat production.

*Nitrogen Elimination.*—The total nitrogen in the urine showed a rise after taking caffein in each experiment except that on J. C. A., when the data were not obtained. This rise was greatest in the first experiment on E. F. D. B., 37 per cent., and next greatest in the second experiment on the same subject, 31 per cent.\* It was least in the case of J. C. F., 6 per cent. J. C. F. likewise had the least rise in basal metabolism, but E. F. D. B. did not have the greatest. This increase might have been merely the washing out of nitrogenous bodies as a result of the caffein diuresis.

*The Pulse Rate.*—This showed sometimes a slight rise, sometimes a slight fall, or else no change at all after the subject received the drug.

#### SUMMARY

An increase of from 7 to 23 per cent. in the basal metabolism was found in four normal subjects after receiving 8 to 10 grains of caffein alkaloid (8.6 mg. per kg. of body weight). After taking the drug there was no significant change in the pulse rate, in the respiratory quotient, in the proportions of the various foodstuffs metabolized, or in the percentage of heat lost in the vaporization of water. The independent methods of direct and indirect calorimetry gave results which agreed within 1 per cent.

477 First Avenue.



## CLINICAL CALORIMETRY

TWENTY-FIRST PAPER

### THE BASAL METABOLISM OF DWARFS AND LEGLESS MEN WITH OBSERVATIONS ON THE SPECIFIC DYNAMIC ACTION OF PROTEIN \*

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WITH THE TECHNICAL ASSISTANCE OF G. F. SODERSTROM

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In order to establish more fully the law that the total body metabolism is proportional to the surface area, it is important to establish the law not only for people of normal size and shape, but also for those who are deformed. If the body surface be a true index of the metabolism, and if the body surface be accurately measured, then dwarfs or men with their legs cut off would show proportionally the same basal metabolism per square meter of surface as do normally shaped individuals. But if this relationship holds, is the increase in heat production which follows the ingestion of large quantities of meat the same as in persons of normal shape? It was to try to answer these questions that this series of dwarfs and legless men were studied in the Sage calorimeter at Bellevue Hospital.

#### THE BASAL METABOLISM OF MEN OF UNUSUAL SHAPE

*History.*—The basal metabolism of normal individuals has been well established by the large number of normal controls gathered by Benedict, Emmes, Roth and Smith;<sup>1</sup> by Means<sup>2</sup> and by Gephart and Du Bois.<sup>3</sup> The total metabolism of dwarfs has been investigated in one case each by Rubner<sup>4</sup> and by McCrudden and Lusk.<sup>5</sup>

Rubner studied a very small dwarf who was 20 years old and

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\* Submitted for publication Dec. 26, 1916.

\* From the Russell Sage Institute of Pathology, in affiliation with the Second Medical Division, Bellevue Hospital.

1. Benedict, F. G., Emmes, L. E., Roth, P., and Smith, H. M.: The Basal Gaseous Metabolism of Normal Men and Women, *Jour. Biol. Chem.*, 1914, **18**, 139.

2. Means, J. H.: Basal Metabolism and Body Surface, *Jour. Biol. Chem.*, 1915, **21**, 263.

3. Gephart, F. C., and Du Bois, E. F.: The Determinations of the Basal Metabolism of Normal Men and the Effect of Food, *THE ARCHIVES INT. MED.*, 1915, **15**, Part 2, p. 835.

4. Rubner, M.: *Biologische Gesetze*, Marburg, 1887, p. 10; *Beiträge zur Ernährung im Knabenalter*, Berlin, 1902, p. 45.

5. McCrudden and Lusk: The Metabolism of a Dwarf, *Jour. Biol. Chem.*, 1912-1913, **13**, 447.

weighed but 6.6 kg., and who "behaved like a fully grown man." His observations were not made under conditions of complete rest, and so are not comparable to our findings. Rubner, however, calculated that the metabolism per square meter, using Meeh's formula, was but slightly higher than that of a breast-fed infant of similar weight. "The larger metabolism of the dwarf," he writes, "is explained by his greater activity, while the infant is very quiet . . . and neither stands nor walks." This showed, according to Rubner, that metabolism was dependent on surface area, not on age.

McCrudden and Lusk's case was in a boy of 17 years, 113 cm. tall and weighing 21.3 kg. By the height-weight chart his surface area is, therefore, 0.8 square meters. He was a patient described by C. A. Herter<sup>6</sup> under the diagnosis of intestinal infantilism. This is a condition in which the arrest of growth is associated with a group of symptoms based on chronic intestinal intoxication. It is associated with fair mental development, marked abdominal distention, moderate anemia, but most strikingly by frequent periods of diarrhea with fatty and putrefying stools. No definite history of this patient is given, but it is one of the cases used by Herter in the description of the syndrome. Practically normal urinary findings on the same case are reported by McCrudden.<sup>7</sup> The patient was studied in a small calorimeter in Dr. Lusk's laboratory, and the routine was practically the same as is now followed in the Sage calorimeter. The results are, therefore, directly comparable to those reported in this paper. Five observations were made on the dwarf, but only one was a true basal experiment, while on the other occasions he had small amounts of food before entering the calorimeter. The one basal observation has been summarized in Table 1, and shows a normal metabolism per square meter of surface area.

In this series we wish to report the metabolism of five dwarfs; two of the achondroplastic type, one rachitic and two with involvement of the glands of internal secretion. Added to this group are two men who had lost both legs. The basal observations in the calorimeter were made in the routine way followed in this laboratory, and fully described in the third and fourth papers of this series.<sup>8</sup> The subjects had had no food for at least fifteen hours, and during the observation remained quietly lying on a bed.

6. Herter, C. A.: On Infantilism from Chronic Intestinal Infection, 1908.

7. McCrudden: Chemical Studies on Intestinal Infantilism, *Jour. Exper. Med.*, 1912, **15**, 107. McCrudden and Fales: *Ibid.*, 1912, **15**, 113.

8. Gephart, F. C., and Du Bois, E. F.: The Organization of a Small Metabolism Ward, *THE ARCHIVES INT. MED.*, 1915, **15**, 829. Determination of the Basal Metabolism of Normal Men and the Effect of Food, *ibid.*, p. 834.

We have followed Rischbieth's<sup>9</sup> classification of dwarfs, so careful description of types will not be discussed here. The differentiation of the rachitic, the achondroplastic and the ateliotic (the perfectly proportioned) types is not difficult with the help of the Roentgen ray. However, we have been puzzled in the distinction between the myxedematous and true dwarfism (or ateliotic) types. The skeleton of the ateliotic dwarfs has the same characteristics of bone formation as has the mild cretin, for the cartilage disks of the long bones (according to Rischbieth, and Sternberg<sup>10</sup>) persist throughout life, and the square shaped skull and cretinoid facial appearance may also be present. Both have defective sexual development and both may be mentally defective. Brissaud<sup>11</sup> classifies myxedema, ateliosis and infantilism in one group and Sternberg admits that this is a possible classification. This difficulty in diagnosis confronted us several times in choosing suitable subjects for the investigation.

#### CASE HISTORIES

**CASE 1.**—Raphael De P. (Figs. 1 and 2), achondroplastic dwarf, born in Italy, aged 35; a kitchen helper; height 134.7 cm.; weight 40.9 kg.

*History.*—The family history is negative; no other dwarf in the family for three generations. Had severe illness when a few months old. Since then always well except for chancroid infection twelve years prior to the test. Has been married for eight years to a woman of normal size; has two children, both of normal size.

*Physical Examination.*—An active, cheerful Italian of normal mentality. The physical examination is negative save for the extremities. All the long bones seem shortened, with slight outward bowing and proportionally large epiphyses. The thighs and upper arms are particularly short. The muscles are powerful. Hands are flat and short, fingers are fat and stubby. Urine, negative. Blood pressure: systolic 120 mm.; diastolic, 90 mm.

**CASE 2.**—George F.,\* hypopituitarism; myxedema; born in Ireland, aged 48; single; a clerk; height 148.8 cm.; weight 53.1 kg.

*History.*—The family history is negative. He has always been small in stature, though active. For six years he has had occasional attacks of edema, particularly about the eyes, feet and hands. For three years he has had slight shortness of breath and failing vision. He sleeps a great deal and perspires very little. He has had no sexual power for many years.

*Physical Examination.*—A short, rather fat individual with cretinoid face and feminine type of body. The genitalia are small, the breasts are large. There is no hair except on the head and eyebrows, and this is dry and slightly coarse.

9. Rischbieth, H., and Barrington, A.: Dwarfism, Treasury of Human Inheritance, London, 1912, Part VII, Sec. xv A.

10. Sternberg, M.: Vegetationsstörungen und Systemerkrankungen der Knochen, Wien., 1899; Nothnagel's specielle Pathologie und Therapie, Vienna, 1903, 7, Part 2.

11. Brissaud, E.: De l'infantilisme myxedemateux, Nuov. iconog. de la Salpêtrière, 1897, 10, 240-282.

\* This patient was carefully studied by Dr. W. M. Krause, who reported the case in the Jour. Nerv. and Ment. Dis., 1917, 45, 193.



The arms are long, the hands of trident type. There was general edema without much pitting on admission, but after a week in the hospital this disappeared, leaving a yellowish, dry, much wrinkled skin. Mentally, he is like a well-behaved child and is always good-natured.

*Laboratory Findings.*—Blood, normal. Urine occasionally showed small amounts of albumin and rare granular casts; 4,000 c.c. of water intake daily for ten days caused no increase in weight. Phenolsulphonephthalein test, 55 and 63 per cent. in two hours. Wassermann negative. Glucose sugar tolerance,



Figure 1



Figure 2

Fig. 1.—Raphael deP. Achondrodystrophy. The meter stick is opposite the spine.

Fig. 2.—Raphael deP. and a dwarf of ateliotic type.

more than 400 gm. Roentgenogram of hands showed epiphyseal ends of bones to be atrophic. The epiphyseal lines are still visible, particularly the lower ends of the radius and ulna. The head shows a markedly enlarged and somewhat eroded sella turcica.

CASE 3.—Samuel G., achondroplastic dwarf, born in Germany, aged 29; single; an actor; height 123.5 cm.; weight 34.9 kg.

*History.*—The family history is negative. His arms and legs have always been very short for his age. Except for an occasional attack of nausea and vomiting, he is perfectly well. Mentally, he is slow and defective; sexually, he is well developed.

*Physical Examination.*—Negative, save for the extremities. The torso is of normal shape and size; the arms and legs very short. The head is large and square, with a high forehead and a saddle-shaped nose. The hard palate is high. The extremities are very short, particularly the upper arms and legs, but the muscular development is good. The feet and hands are almost normal size and large for the rest of the extremities. The genitalia are of mature development, the body hair is abundant and of normal masculine distribution. The urine is normal.

CASE 4.—Patrick W. (Figs. 3, 4, 5 and 6), rachitic dwarf, born in United States, aged 38; actor; height 123.8 cm.; weight 37.3 kg.



Fig. 3.—Pat W., rachitic dwarf.

Patient says he was normal until 3 years old when he became moody and his body became distorted. He had walked and talked at the usual age. He has had gonorrhea three times; twelve years prior to the test he had syphilis, followed by very severe nephritis. During this attack he was very edematous and the abdomen was tapped eight times. Since then he has felt perfectly well.

*Physical Examination.*—A small, misshapen dwarf with marked curvature of the spine and the longer bones. The head appears normal. The chest is very narrow in the upper portion, with a prominent sternum. There is marked flaring of the lower ribs, with Huntington's groove and marked rosary. The spine shows left lateral curvature in the lower thoracic region, with a slight kyphosis. The legs are very short and markedly bent. The feet appear normal.

The arms are short, but the bones are only slightly curved. The epiphyses of all the long bones are large. The genitalia are well developed; the body hair is of masculine type of distribution.

*Laboratory Findings.*—Urine was negative; specific gravity, 1.025; albumin, negative. Microscopic examination, negative.

CASE 5.—Irwin E., myxedematous dwarf, born in United States, aged 32; actor; height 134 cm.; weight 37.4 kg.

*History.*—He has always been of small size. At 10 years of age he was short and weighed but 40 pounds. His school work was not very good, as he failed four times in grammar school. He passed for high school when he was



Fig. 4.—Pat W.

15 years old, and stayed there only one year. He is sexually mature, but inactive. The hair on his head has always been soft and plentiful. He perspires freely.

*Physical Examination.*—He appears like a well developed boy of 14 years. The facies suggests cretinism—thick eyebrows, puffy eyelids, broad nose, wide maxillae, broad jaw and large, widely spaced teeth. There is a pad of fat between the shoulders and a slight degree of adiposity. The hands are short and stubby; the skin slightly dry, but soft and smooth. The genitalia appear normally developed, but the secondary characteristics are scant for there is no hair on the face, in the axillae or on the body, and the pubic hair is of the feminine configuration. The voice is that of male puberty. Otherwise the



physical examination is not remarkable. Blood pressure: systolic, 95 mm.; diastolic 65 mm.

Roentgenogram of head shows a slightly enlarged sella turcica. In the hand there is some hypo-atrophy of the terminal tufts of the phalanges. The epiphyses of the radius and ulna are not united. The epiphyseal lines of the phalanges are clear.

CASE 6.—Harry J. (Fig. 7, described in Paper 9), a legless man, born in the United States, colored, aged 34; beggar. Length is approximately 103 cm., weight 54.6 kg.



Fig. 5.—Pat W. Roentgenogram of knee.

*History.*—Both legs were cut off by a train when the patient was 6 years old, and since then he has propelled himself on wheels. Seven years prior to the test the right side of the body suddenly became numb and the right arm was completely paralyzed. Slight power returned slowly. He had urethritis seven years previous to the examination, possibly with a chancre, though no secondary manifestations developed.

*Physical Examination.*—Very muscular negro with short leg stumps. *Face:* Forehead is normal but right side of face is paralyzed; mouth droops and tongue deviates slightly to right. *Chest* is unusually broad and deep, with very powerful muscles in the back, neck and left shoulder and arm. The waist is very narrow; the hips narrow, with poorly developed muscles. The right arm is paralyzed and the muscles are of moderate size but flabby. The



Fig. 6.—Pat W. Spine.

reflexes are exaggerated. The right leg is a very short stump with flabby muscles. The left leg is amputated just above the knee and the stump tapers sharply from the perineal level. The muscles are small and weak. Blood pressure: systolic, 188 mm.; diastolic, 130 mm.

*Urine*, negative. *Wassermann*, negative.

CASE 7.—Robert L. (described in Paper 9), a legless man, born in the United States, aged 43; beggar; length about 125 cm.; weight 63.8 kg.

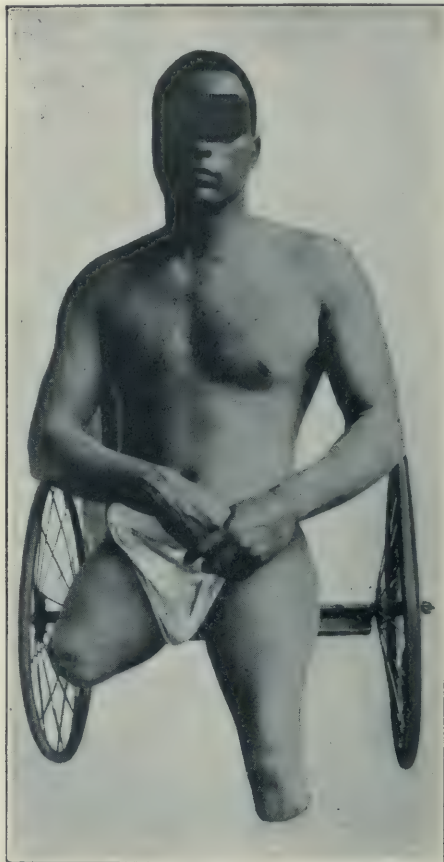


Fig. 7.—Harry J.

*History*.—The patient was a boiler maker for over twenty years and was always healthy and strong. He had syphilis and urethritis nineteen years ago, for which he received treatment for five months. Five years prior to the examination he was hit by a railroad train and both his legs had to be amputated. He now feels well and supports himself by selling pencils. He walks about a mile every day, with crutches.

*Physical Examination*.—A heavily built, healthy looking man with fat cheeks and prominent abdomen. His chest is large; his arms muscular. The left thigh is cut off just below the level of the perineum. The right leg is cut off just below the level of the knee and the thigh muscles are firm and strong. Otherwise, the patient appears normal, except that over the left lung there are heard many dry, crackling râles, though resonance, tactile fremitus, vocal fremitus and breath sounds are normal.



TABLE 1.—SUMMARY

Case Number and Name	Subject	Age	Height, Cm.	Weight, Kg.	Surface Area, Sq. M. Linear Formula	Calories per Sq. M. per Hour	Variations from Average Normal 39.7 %	Average R. Q.	Total Calories Measured		Divergence of Direct from Indirect, Per Cent.	Average Calories per Hour, Indirect
									Indirect	Direct		
6. Harry J.* .....	Legless man	35	103±	54.61	1.34	41.3	+4.0	0.80	110.67	106.42	-3.9	55.94
7. Robert L. ....	Legless man	43	125±	63.81	1.43	41.3	+4.0	0.80	176.90	178.22	+0.7	58.97
4. Patrick W. ...	Rachitic dwarf	38	123.8	37.31	1.20	41.0	+3.0	0.81	98.27	97.74	-0.5	49.17
1. Raphael DeP.	Achondroplasia	35	134.7	40.86	1.24	42.2	+6.3	0.81	104.66	102.20	-2.3	52.33
3. Samuel G.†...	Achondroplasia	29	123.5	34.92	1.08	(48.9)	(+25.0)	(0.82)	(39.58)	(38.26)	-3.3	52.76
5. Irwin E. ....	Myxedema	32	134.0	37.37	1.17	29.5	-25.7	0.85	68.98	70.31	+1.9	34.49
2. George F. ....	{ Hypopituitary Hypothyroid	48	148.9	53.05	1.51	31.1	-22.0	0.83	96.57	94.51	-2.1	48.28
J P.‡.....	{ "Intestinal Infantilism"	17	113.3	21.3	0.8	38.2	-4.0	0.79	61.12			

\* Quiet experiment, 5/6/16.  
† Forty-five-minute period only.  
‡ McCrudden and Lusk's case.  
Nauseated. Other observations two hours.

## DISCUSSION OF RESULTS

Two of the experiments here recorded were technically unsatisfactory and cannot be considered as giving accurate evidence as to the level of the basal metabolism. The achondroplastic dwarf, Samuel G., was nauseated in the calorimeter and the high figures obtained in his brief observation might have been due to the same factor which caused his vomiting. The legless negro, Harry J., was also restless the first time he was in the calorimeter. While he did not move often, his deformity and paralysis of the right arm made each movement a great exertion. In his second experiment he was quiet and the results were satisfactory.

This leaves good determinations of the basal metabolism of seven men of unusual body shape. Five give figures within the normal limits when the results are calculated according to the surface area. These individuals are the two legless men, Harry J. and Robert L., the rachitic dwarf, Patrick W., the achondroplastic dwarf, Raphael de P., and the dwarf, J. P., with intestinal infantilism, described by McCrudden and Lusk. Two dwarfs give results distinctly below the normal. These are Irwin E. and George F., both of whom had many of the characteristics of cretinism, a condition which is always accompanied by a diminution in the heat production.

It therefore seems true that people with abnormal body shape, but with normal glands of internal secretion, have a metabolism which conforms to their surface area. It is therefore possible that the height of metabolism may be a method for differentiating the ateliotic and myxedematous dwarfs.

## THE SPECIFIC DYNAMIC ACTION OF PROTEIN

Many investigators have studied the increased heat production which accompanies the metabolism of protein. The literature has been thoroughly reviewed and discussed by Lusk<sup>12</sup> in his book on nutrition, so that only the most important references are given here.

Von Mering and Zuntz<sup>13</sup> believed that the increased metabolism was due to intestinal work following ingestion of food. This was disproved, however, by the fact that fat and carbohydrate increase metabolism far less than an equal quantity of protein. Rubner<sup>14</sup> and Magnus-Levy<sup>15</sup>

12. Lusk, G.: *The Elements of the Science of Nutrition*, Ed. 3, Philadelphia, 1917.

13. Von Mering and Zuntz, N.: In wiefern beeinflusst Nahrungszufuhr die thierischen Oxydationsprocesse, *Pflüger's Arch. f. d. ges. Physiol.*, 1877, **15**, 634.

14. Rubner, Max: *Die Gesetze des Energieverbrauchs bei Ernährung*, Leipzig, 1902.

15. Magnus-Levy, Ad.: Ueber die Grösse des respiratorischen Gaswechsels unter dem Einfluss der Nahrungsaufnahme, *Pflüger's Arch. f. d. ges. Physiol.*, 1894, **55**, 1.

fed bones to a dog and obtained but slight increase in metabolism in spite of the intestinal irritation and activity. Rubner<sup>14</sup> believed that this "specific dynamic action" was due to the cleavages and oxidations necessary to make the foodstuffs available for use by the body cells. The heat given off by these preliminary reactions constituted the waste heat of the dynamic action. In the case of protein, part of the heat, he thought, was derived from the carbohydrate portion burned, but chiefly it arose from the chemical reactions of the remainder of the protein molecule, whose end-products appear in the urine, mostly as urea. This energy, he thought, was liberated unused by the organism. Voit<sup>16</sup> believed that the cells of the body were stimulated to a higher metabolism by food being brought to them. Lusk<sup>17</sup> gave amino-acids and glucose to normal and phlorhizinized dogs. The metabolism of the normal dog was stimulated by both foodstuffs, but the phlorhizinized dog had no change in metabolism when glucose was administered and totally excreted. However, the amino-acids, glycolic acid and alanin, though unoxidized and excreted with all their energy as sugar and urea, still caused an increase of metabolism. The conclusion is clear that it is not the energy contents of the amino-acids themselves, for those were excreted, but, to quote from the original, "that intermediary products, such as glycolic acid or lactic acid, provide the stimulus. These experiments afford conclusive proof of a true chemical stimulation of protoplasm within the mammalian organism, and offer a logical explanation of the specific dynamic action of protein."

It was to find out where this chemical stimulation occurred, whether in the muscles or in the internal organs, that the following experiments were made at the suggestion of Dr. Lusk. Individuals were taken with normal torsos, but a marked variation in the size of their extremities. If the specific dynamic action of a test protein meal were due to the muscle volume or the surface area, then an achondroplastic dwarf and legless man should show a proportionally smaller stimulation of metabolism than did the normal controls. If the action were due to one of the internal organs, the response of all the subjects tested should be approximately the same.

The subjects chosen were a legless man, an achondroplastic dwarf and three normal controls whose weight closely approximated that of the two abnormal cases. The basal metabolism was determined, following the usual routine technic of this laboratory, fifteen to eighteen hours after the last food. Within the next week the subject was prepared as for a basal observation, but in the morning he was given a

16. Voit, C.: *Physiologie des Stoffwechsels und der Ernährung*, Leipzig, 1881, p. 308.

17. Lusk, G.: *Animal Calorimetry*, XI, An Investigation Into the Causes of the Specific Dynamic Action of the Foodstuffs, *Jour. Biol. Chem.*, 1915, **20**, 555.



large protein meal. This consisted in all cases, except that of Louis M., of about 660 gm. of thoroughly scraped lean beefsteak, chopped very fine and cooked only superficially. In each case a sample was analyzed for protein and fat. A small amount of stewed tomatoes and "Kaffee Hag" were allowed in order to make the food more palatable. Louis M., whose observations are taken from the work of Gephart and Du Bois,<sup>18</sup> had been given the same meal with the addition of 100 gm. of fat. When the subject had finished eating he was put in the calorimeter, and two hours after the start of the meal the observation was begun. In several cases the metabolism was also determined for a thirty-minute period, starting one and one-half hours after the start of the meal. All of the subjects received approximately the same amount of protein (24 gm. of nitrogen) and the temperature of the calorimeter was the same for all observations, so that the results are directly comparable; for Rubner<sup>14</sup> pointed out that a change in temperature has a marked effect on the specific dynamic action of protein, and Gigon<sup>19</sup> claimed that the dynamic action did not vary directly with the amount of protein ingested.

The urine was collected in hourly periods, when this was possible, and each sample was analyzed separately for nitrogen, and in most observations for sulphur. In this way the speed of the nitrogen and sulphur excretion could be compared with the increase in metabolism (Table 2). The urinary nitrogen was determined by the usual Kjeldahl method and the sulphur by Benedict's method. The amount of protein and fat ingested was calculated from analyses of each sample of steak used.

#### CASE HISTORIES

CASE 8.—S. K., normal control, born in Japan, aged 33; medical student; height 161.5 cm.; weight 49.5 kg.

The history is entirely negative, except that fifteen years prior to examination he had slight symptoms of beriberi, which rapidly disappeared when he changed his diet. He exercises a great deal, mostly at jiu jitsu and wrestling.

The physical examination is negative. He is a Japanese of very marked muscular development and good physique.

CASE 9.—G. T. B., normal control, born in the United States, aged 25; medical student; height 164.5 cm., weight 54.4 kg.

He has always been fairly well, but never athletic or very strong. His life has been a sedentary one, particularly for some months previous to the test, during which he has been studying for examinations.

The physical examination is negative.

CASE 10.—Louis M.,<sup>18</sup> normal control, born in Germany, aged 22 years; barber; weight 51.7 kg.

A short, thin, normal individual with small frame and muscles.

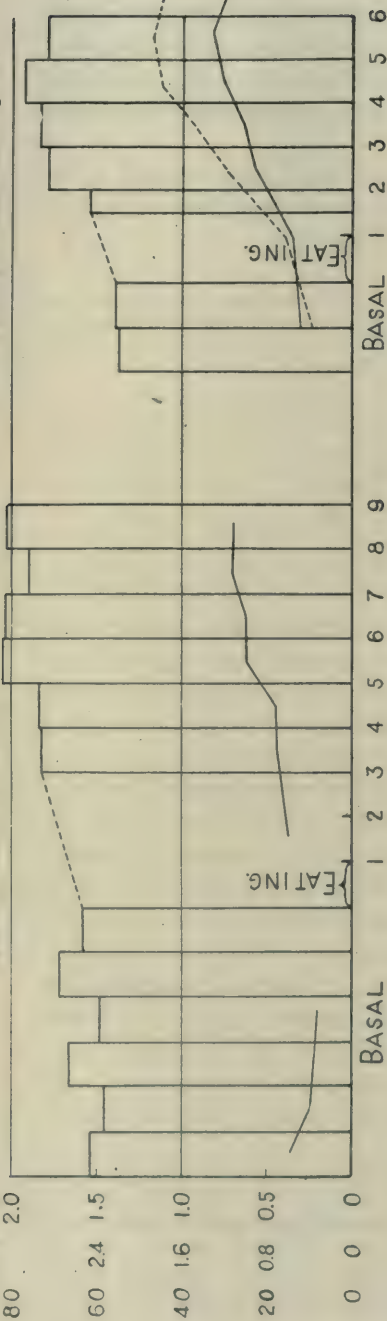
18. Gephart, F. C., and Du Bois, E. F.: Determination of the Basal Metabolism of Normal Men and the Effect of Food, *Clinical Calorimetry*, Fourth Paper, *THE ARCHIVES INT. MED.*, 1915, **15**, 835.

19. Gigon, Alfred: Ueber den Einfluss der Nahrungsaufnahme auf den Gaswechsel, *Arch. f. Physiol.*, 1911, **140**, 544.

CAL-N-S-  
80 2.0

NORMAL CONTROL - L.M.

NORMAL CONTROL - S.K.



CAL-N-S-  
80 2.0

NORMAL CONTROL - W.B.

ACHONDROPLASIA - R.D.P.

LEGLESS MAN - H.J.

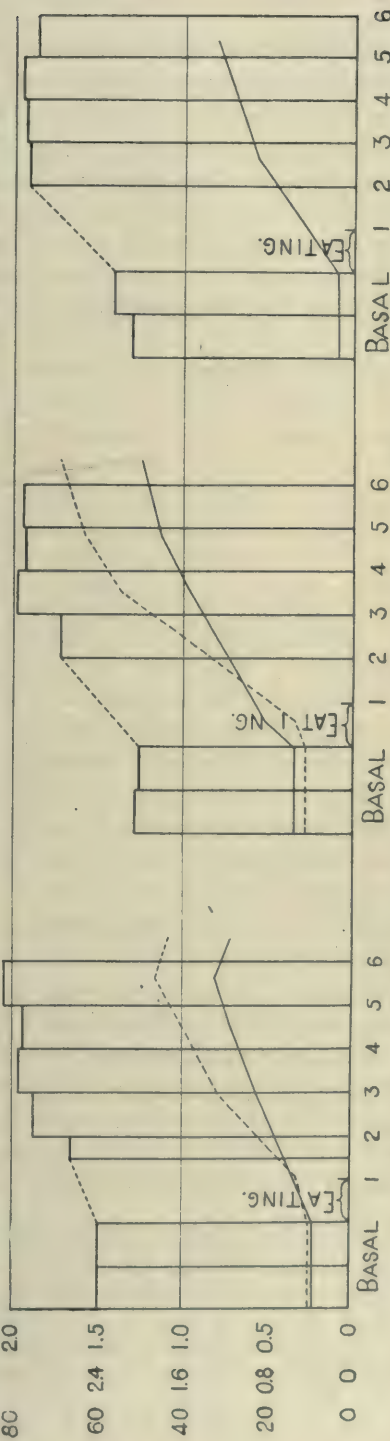


Chart 1.—Specific dynamic action of protein. Columns show the basal heat production in calories per hour and the increased metabolism after the subject has eaten chopped beef containing 23 to 25 gm. of nitrogen. The dotted line represents the excretion of sulphur in the urine in decigrams. The continued line gives the nitrogen elimination in grams.

The observations brought out several points of interest. The graphic chart shows that the metabolism had risen to a considerable degree one and one-half hours after the meal, and nearly to the maximum intensity two hours after eating. It remained at the high level for at least seven hours, with the maximum heat production between the fourth and sixth hours following ingestion of food. This shows that the absorption of food from the intestine is rapid, for certainly within one and one-half hours after partaking of food it has been partly absorbed from the bowel and is being metabolized.

TABLE 2.—NITROGEN AND SULPHUR EXCRETION IN THE DOG (AFTER RUBNER)

Period	Sulphur	Nitrogen	$\frac{N}{S}$
9-3	0.448	5.57	12.4
3-9	0.387	8.94	23.1
9-3	0.257	5.32	20.7
3-9	0.131	2.66	20.3

But what is the index of the true protein metabolism? Voit emphasized the fact that the amount of protein eaten was not good evidence of the amount metabolized, but that the urinary nitrogen was a much more direct index. But even though the urinary nitrogen is direct evidence, its hourly value is still rising for some hours after the stimulation of protoplasm has reached its maximum. This same fact has been observed in the study of dogs by Williams, Riche and Lusk,<sup>20</sup> who point out that there is a delay in the excretion of nitrogen following the metabolism of protein. They found that after giving 700 gm. of meat to the dog the maximum increase of metabolism was reached in two hours, but that the urinary nitrogen excretion did not reach its maximum until the sixth hour. There is, during this time, a temporary accumulation of nonprotein nitrogen, largely of the urea portion, in the blood and tissues, as was shown by Folin and Denis<sup>21</sup> and by Miss Wishart.<sup>22</sup> It was also shown by Reilly, Nolan and Lusk<sup>23</sup> that in a dog made diabetic with phlorhizin, the sugar derived from the

20. Williams, Riche and Lusk: Metabolism of the Dog Following the Ingestion of Meat in Large Quantities, *Jour. Biol. Chem.*, 1912, **12**, 349.

21. Folin, Otto, and Denis, W.: Protein Metabolism from the Standpoint of Blood and Tissue Analysis, *Jour. Biol. Chem.*, 1911-1912, **11**, 87.

22. Wishart, Mary: Animal Calorimetry, Paper IX. The Influence of Meat Ingestion on the Amino-Acid Content of Blood and Muscle, *Jour. Biol. Chem.*, 1915, **20**, 535.

23. Reilly, F. H., Nolan, F. W., and Lusk, G.: Phlorhizin Diabetes in Dogs, *Am. Jour. Physiol.*, 1898, **1**, 395.



protein was excreted more rapidly than was the nitrogen. This is direct proof of the delayed excretion of nitrogen after its metabolism. Lusk<sup>24</sup> and Csonka<sup>25</sup> have shown that the administration of amino-acids to the phlorhizinized dog causes a maximal rise in both the heat production and sugar elimination in the first one or two hours, which indicates

TABLE 3.—NITROGEN-SULPHUR RATIOS AFTER MEAT INGESTION

Name	Date	Time	Nitrogen per Hour, Gm.	Sulphur per Hour, Mg.	N — S	Remarks
R. DeP.	3/15	7:20 a. m. to 1:10 p. m.	0.60	.....	....	Basal
	3/16	5:00 a. m. to 8:55 a. m.	0.57	31.9	17.7	662 gm. meat at 9:00 a. m.
		8:55 a. m. to 9:55 a. m.	0.87	35.3	24.7	
		9:55 a. m. to 12:17 p. m.	1.27	82.6	15.7	
		12:17 p. m. to 1:32 p. m.	1.61	135.6	11.9	
		1:32 p. m. to 3:04 p. m.	1.88	159.7	11.8	
		3:04 p. m. to 4:02 p. m.	2.02	173.0	11.7	
S. K.	5/16	9:27 a. m. to 1:26 p. m.	0.46	22.7	20.2	Basal
	5/18	9:15 a. m. to 11:23 a. m.	0.55	38.8	14.2	660 gm. meat at 8:20 a. m.
		11:23 a. m. to 12:22 p. m.	0.91	74.8	12.2	
		12:22 p. m. to 1:23 p. m.	1.02	94.1	10.9	
		1:23 p. m. to 2:32 p. m.	1.19	111.8	10.1	
		2:32 p. m. to 3:30 p. m.	1.29	117.9	10.9	
		3:30 p. m. to 4:24 p. m.	1.19	111.7	10.6	
W. B.	5/23	8:45 a. m. to 1:02 p. m.	0.37	25.0	14.6	Basal
	5/25	6:55 a. m. to 8:23 a. m.	0.54	32.7	16.6	600 gm. meat at 8:25 a. m.
		8:23 a. m. to 10:29 a. m.	0.61	34.4	17.7	
		10:29 a. m. to 12:30 p. m.	0.92	80.8	11.4	
		12:30 p. m. to 1:30 p. m.	1.12	104.3	10.7	
		1:30 p. m. to 2:28 p. m.	1.22	118.2	10.3	
		2:28 p. m. to 3:29 p. m.	1.13	110.7	10.2	
Harry J.	5/10	5:00 a. m. to 9:46 a. m.	0.38	25.7	14.7	660 gm. meat at 9:00 a. m.
		9:46 a. m. to 1:48 p. m.	0.94	61.1	15.3	
		1:48 p. m. to 3:07 p. m.	1.30	114.5	11.3	

that the rise in heat production approximately parallels the speed of amino-acid metabolism. This is before the period of maximal nitrogen elimination. Then, too, the sulphur portion of the protein is excreted

24. Lusk, G.: Animal Calorimetry, Paper XI. An Investigation Into the Causes of the Specific Dynamic Action of the Foodstuffs, Jour. Biol. Chem., 1915, **20**, 610.

25. Csonka, F. A.: Animal Calorimetry, Paper X. The Rate at Which Ingested Glycocoll and Alanine Are Metabolized, Jour. Biol. Chem., 1915, **20**, 539.

TABLE 4.—DATA OF CALORIMETER—

Subject, Date, Weight, Surface Area, Linear Formula	Period	End of Period	Carbon Dioxid, Gm.	Oxygen, Gm.	R. Q.	Water, Gm.	Urine N per Hour, Gm.	Indirect Calo- rimetry, Cal.	Heat Elimi- nated, Cal.
Case 3 (Saml. G.)..	Prelim.	10:14	.....	.....	.....	.....	.....	.....	.....
3/8/16	1	10:50	13.4	11.9	0.82	19.2	0.261	39.6	40.2
34.9 Kg.									
1.08 Sq. M.									
Case 4 (Pat W.)...	Prelim.	11:38	.....	.....	.....	.....	.....	.....	.....
3/24/16	1	12:38	15.7	13.7	0.83	16.1	0.266	46.0	47.1
37.3 Kg.	2	1:38	17.1	15.8	0.79	17.3	0.266	52.3	50.1
1.20 Sq. M.	Aver.	.....	.....	.....	.....	.....	.....	.....	.....
Case 5 (Erwin E.)..	Prelim.	12:05	.....	.....	.....	.....	.....	.....	.....
3/6/16	1	1:05	12.1	10.6	0.83	12.5	0.314	35.3	40.6
37.4 Kg.	2	2:05	11.9	10.0	0.86	12.5	0.314	33.7	38.0
1.17 Sq. M.	Aver.	.....	.....	.....	.....	.....	.....	.....	.....
Case 2 (Geo. F.)...	Prelim.	11:40	.....	.....	.....	.....	.....	.....	.....
1/7/16	1	12:40	16.4	14.0	0.86	16.5	0.309	47.0	51.3
53.1 Kg.	2	1:40	16.2	15.0	0.79	16.2	0.309	49.6	50.6
1.51 Sq. M.	Aver.	.....	.....	.....	.....	.....	.....	.....	.....
Case 8 (S. K.).....	Prelim.	10:50	.....	.....	.....	.....	.....	.....	.....
5/12/16	1*	11:20	11.5	9.6	0.87	13.0	1.288	31.7	32.5
50.4 Kg.	2	12:20	26.1	21.2	0.89	23.2	1.288	70.6	66.5
1.50 Sq. M.	3	1:20	25.3	22.1	0.83	27.4	1.288	72.3	71.3
	4†	2:30	28.9	25.9	0.81	35.5	1.288	84.4	89.1
	5†	3:20	19.7	18.6	0.77	25.4	1.288	59.9	62.5
S. K. ....	Prelim.	10:56	.....	.....	.....	.....	.....	.....	.....
5/16/16	1	11:56	18.3	16.5	0.81	23.3	0.457	54.8	61.6
49.5 Kg.	2	12:56	17.9	16.9	0.77	26.1	0.457	55.5	61.2
1.50 Sq. M.	Aver.	.....	.....	.....	.....	.....	.....	.....	.....
Case 7 (Robt. L.)..	Prelim.	11:21	.....	.....	.....	.....	.....	.....	.....
12/9/14	1	12:21	18.2	16.0	0.83	23.5	0.536	53.0	57.8
63.8 Kg.	2	1:21	21.1	19.0	0.81	24.9	0.536	63.0	65.3
1.43 Sq. M.	3	2:21	19.8	18.6	0.77	24.3	0.536	60.9	64.0
(measured)	Aver.	.....	.....	.....	.....	.....	.....	.....	.....
Case 6 (Harry J.)..	Prelim.	11:06	.....	.....	.....	.....	.....	.....	.....
12/11/14	1	12:06	20.7	18.8	0.80	23.7	0.419	62.5	59.3
55.8 Kg.	2	1:06	22.1	18.1	0.89	25.2	0.298	61.6	62.0
1.33 Sq. M.	3	2:06	21.9	19.9	0.80	24.5	0.298	66.2	61.3
	Aver.	.....	.....	.....	.....	.....	.....	.....	.....

\* 30-minute period.

† 70-minute period.

‡ 50-minute period.

## EXPERIMENTS IN DWARFS AND LEGLESS MEN

Direct Calorimetry (Rectal Temp.), Cal.	Rectal Temp., C.	Average Pulse	Work-Adder, Cm.	Non-protein R. Q.	Per Cent. Calories from			Calories per Hour		Remarks
					Protein	Fat	Carbo-hyd.	Per Kg.	Per Sq. M. (Lin.)	
.....	36.9	80	.....	.....	..	..	..	....	....	Basal; in bed
38.3	36.9	80	11	0.82	18	49	33	1.51	48.9	Quiet, 45 min. period; at 11:32 became nauseated
.....	36.6	.....	.....	.....	..	..	..	....	....	Basal; in bed
46.9	36.6	70	15	0.84	15	48	37	....	....	Asleep 30 minutes; very quiet
50.9	36.6	79	17	0.79	14	63	23	....	....	Awake; quiet
.....	.....	.....	.....	.....	15	55	30	1.32	41.0	
.....	37.3	.....	.....	.....	..	..	..	....	....	Basal; in bed
35.0	37.2	61	11	0.84	..	..	..	....	....	{ Very quiet; asleep 30 min.; read 10 min. Quiet; no reading
35.3	37.1	64	7	0.88	..	..	..	....	....	
.....	.....	.....	.....	0.86	24	36	40	0.92	29.5	
.....	37.0	.....	.....	.....	..	..	..	....	....	Basal; in bed
47.1	36.9	59	15	0.87	17	37	46	....	....	Quiet; dozed
47.4	36.8	59	16	0.79	17	61	22	....	....	Quiet
.....	.....	.....	.....	0.83	17	49	34	0.89	31.1	
.....	36.8	.....	.....	.....	..	..	..	....	....	{ 9:15 to 10:12, ate 602 gm. beef containing 24.1 gm.N. Very quiet
29.9	36.7	67	4	0.95	54	8	38	....	....	
66.8	36.7	.....	12	0.99	48	2	50	....	....	Very quiet
69.5	36.7	69	18	0.86	47	25	28	....	....	Very quiet
88.3	36.7	69	26	0.82	47	32	21	....	....	Quiet
61.4	36.7	65	.....	0.74	48	46	6	....	....	Quiet
.....	36.4	.....	.....	.....	..	..	..	....	....	Basal; in bed
53.9	36.2	55	3	0.81	..	..	..	....	....	Very quiet; almost motionless
60.9	36.2	55	9	0.76	..	..	..	....	....	Very quiet; urinated
.....	.....	.....	.....	.....	22	57	21	1.11	36.8	
.....	36.9	.....	.....	.....	..	..	..	....	....	Basal
51.7	36.8	78	8	0.84	27	40	33	....	....	Asleep
63.2	36.8	72	22	0.81	23	50	27	....	....	Fairly quiet
63.4	36.8	73	19	0.76	23	62	15	....	....	Fairly quiet
.....	.....	.....	.....	.....	..	..	..	....	41.3	
.....	36.8	.....	.....	.....	..	..	..	....	....	"Basal"
53.4	36.7	64	13	0.80	18	56	26	1.12	....	Restless
62.0	36.7	65	16	0.91	13	27	60	1.10	....	Restless
62.2	36.8	62	13	0.80	12	60	28	1.10	....	Restless



TABLE 4.—DATA OF CALORIMETER EXPERIMENTS—

Subject, Date, Weight, Surface Area, Linear Formula	Period	End of Period	Carbon Dioxid, Gm.	Oxygen, Gm.	R. Q.	Water, Gm.	Urine N per Hour, Gm.	Indirect Calo- rimetry, Cal.	Heat Elimi- nated, Cal.
Harry J. .... 5/5/16 54.6 Kg. 1.34 Sq. M.	Prelim.	10:36	.....	.....	.....	.....	.....	.....	.....
	1	11:36	17.8	15.9	0.82	25.6	0.210	53.1	54.2
	2	12:36	18.5	17.4	0.78	24.8	0.210	57.5	55.0
	Aver.	.....	.....	.....	.....	.....	.....	.....	.....
Harry J. .... 5/10/16 55.8 Kg. 1.34 Sq. M.	Prelim.	11:00	.....	.....	.....	.....	.....	.....	.....
	1	12:00	26.2	23.9	0.80	28.9	1.298	77.9	69.3
	2	1:00	27.1	23.9	0.83	33.7	1.298	78.2	74.8
	3	2:00	27.8	24.0	0.84	40.6	1.298	78.9	81.3
	4	3:00	26.3	23.1	0.83	43.9	1.298	75.6	80.3
Case 1. .... (Raphael DeP.) 3/15/16 40.9 Kg. 1.24 Sq. M.	Prelim.	11:02	.....	.....	.....	.....	.....	.....	.....
	1§	12:05	18.9	16.8	0.82	22.5	0.603	52.8¶	54.0
	2¶	1:02	16.6	15.0	0.81	20.8	0.603	51.9¶	50.6
	3	.....	.....	.....	.....	.....	.....	.....	.....
Raphael DeP. .... 3/16/16 40.9 Kg. 1.24 Sq. M.	Prelim.	11:02	.....	.....	.....	.....	.....	.....	.....
	1	12:02	24.6	21.6	0.83	27.8	1.27 (1.88)**	70.8 (69.6)**	63.7
	2	1:02	28.4	24.6	0.84	43.6	1.61 (1.88)	80.5 (79.9)	78.9
	3	2:02	27.1	24.2	0.82	50.6	1.75 (1.88)	78.2 (77.9)	82.6
	4	3:02	26.1	24.5	0.77	50.7	1.88 (1.88)	78.2 (78.2)	81.4
	5	4:02	.....	.....	.....	.....	2.02	.....	.....
Case 9 (G. T. B.).. 5/23/16 54.4 Kg. 1.56 Sq. M.	Prelim.	11:04	.....	.....	.....	.....	.....	.....	.....
	1	12:04	20.8	18.3	0.83	29.5	0.380	61.1	60.6
	2	1:04	20.3	17.9	0.83	28.5	0.380	59.8	63.2
	Aver.	.....	.....	.....	.....	.....	.....	.....	.....
G. T. B. .... 5/25/16 55.5 Kg. 1.56 Sq. M.	Prelim.	9:55	.....	.....	.....	.....	.....	.....	.....
	1	10:25††	12.4	9.9	0.91	22.6	1.219	33.7	33.3
	2	11:25	27.2	22.8	0.87	42.1	1.219	75.7	76.9
	3	12:25	27.4	24.0	0.83	40.4	1.219	79.0	75.7
	4	1:25	26.7	23.8	0.82	37.4	1.219	78.0	79.3
	5	2:25	27.9	24.9	0.81	40.6	1.219	81.7	84.5

\*\* Figures in brackets based on assumption that the maximum nitrogen excretion represents true protein metabolism for whole experiment.

§ 63-minute period.

¶ Per hour.

¶ 57-minute period.

†† 30-minute period.

## —IN DWARFS AND LEGLESS MEN—(Continued)

Direct Calorimetry (Rectal Temp.), Cal.	Rectal Temp., C.	Average Pulse	Work-Adder, Cm.	Non-protein R. Q.	Per Cent. Calories from			Calories per Hour		Remarks
					Protein	Fat	Carbohyd.	Per Kg.	Per Sq. M. (Lin.)	
.....	.....	....	.....	.....	..	..	..	....	47.7	{ Excluded from averages on account of restlessness Basal; in bed
.....	36.7	60	.....	.....	..	..	..	....	....	
49.9	36.6	58	7	0.82	..	..	..	....	....	Very quiet; asleep 30 minutes
56.5	36.7	60	7	0.77	..	..	..	....	....	Very quiet
.....	.....	....	.....	0.79	10	68	27	1.01	41.3	
.....	36.9	....	78	.....	..	..	..	....	....	{ 8:30 to 9:50 a. m. ate 660 gm. beef containing 24.6 gm. N.
68.5	36.9	14	76	0.79	44	40	16	....	58.1	Fairly quiet
76.3	36.9	16	83	0.85	44	29	27	....	58.4	Fairly quiet
84.9	37.0	22	84	0.88	44	24	32	....	59.0	Fairly quiet; voided
79.7	37.0	13	83	0.85	46	28	26	....	56.4	Quiet
.....	36.5	....	.....	.....	..	..	..	....	....	Basal
52.5	36.5	68	1	0.83	..	..	..	....	....	Awake; motionless
49.7	36.4	64	1	0.81	..	..	..	....	....	Awake; motionless
.....	.....	....	.....	0.82	31	43	26	1.28	42.2	{ 9:00 to 10:05 a. m. ate 662 gm. beef containing 23.2 gm. N.
.....	36.9	....	.....	.....	..	..	..	....	....	Asleep 60 minutes. Motionless
71.7	37.1	77	4	0.85 (0.88)**	47 (72)**	28 (11)**	25 (17)**	1.72 (1.69)	57.1 (56.1)	Awake; very quiet
78.8	37.2	83	12	0.88 (0.90)	53 (63)	19 (12)	28 (25)	1.96 (1.94)	65.0 (64.5)	Awake; very quiet
81.6	37.2	84	12	0.83 (0.84)	59 (64)	28 (20)	18 (16)	1.80 (1.89)	63.0 (62.8)	Awake; very quiet
78.1	37.1	79	10	0.72 (0.72)	64 (64)	34 (34)	2 (2)	1.90 (1.90)	63.0 (63.0)	Awake; very quiet
.....	36.8	....	.....	.....	..	..	..	....	....	Basal; normal control
63.9	36.9	58	6	0.83	..	..	..	....	....	Almost motionless
63.3	36.9	58	8	0.83	..	..	..	....	....	Almost motionless
.....	.....	....	.....	.....	17	47	36	1.11	38.7	{ 8:30 to 9:15 a. m. ate 600 gm. beef containing 23.7 gm. N.
.....	36.9	....	.....	.....	..	..	..	....	....	Motionless
34.0	36.9	60	0	0.94	24	15	61	....	....	Almost motionless
75.4	37.0	66	12	0.92	43	17	40	....	....	Almost motionless
80.3	37.1	64	17	0.85	41	30	29	....	....	Almost motionless
77.5	37.1	64	17	0.82	42	35	23	....	....	Almost motionless
85.2	37.1	72	17	0.82	40	37	23	....	....	Almost motionless

more rapidly than the nitrogen, as first shown in dogs by Rubner<sup>26</sup> and in humans by Wolf.<sup>27</sup> Rubner's figures were taken in six-hour periods and show how long continued is the high nitrogen excretion after a meat diet containing 24.7 gm. nitrogen.

The sulphur elimination is shown in Table 2 to be very much more rapid. One would expect the nitrogen to sulphur ratio to be 16, which is that found in meat, but because of the variation in speed of excretion, the ratio falls during the first six hours and is too high in the following periods.

Our observations, which are made in very much shorter periods and extend only to seven hours after food, confirm these findings, except that in the hour during the ingestion of food the nitrogen excretion rises markedly; the sulphur increases but little. This is probably due to the rapid elimination of the extractives in the meat.

This delayed elimination of nitrogen after marked protein ingestion makes its hourly excretion a poor index of the metabolism during that hour. Since the total metabolism stays at a nearly constant level throughout the observation after food, it seems probable that the protein metabolism also stays at a constant level. The same value ought, therefore to be used throughout the observation, not a different one for each hour. The best value to use seems to be the maximum hourly nitrogen excretion and this has been used in all calculations. The effect of this choice is but slight in regard to the height of the total hourly metabolism, but the percentage of calories derived from the three food-stuffs is markedly affected, particularly in the early hours of the observation. This effect can be seen in Table 4, in which, in the case of R. De P., the calculations are made in both ways: (1), using the N as excreted; and (2), using the maximum value as the hourly excretion. The figures for Louis M. will be found in Paper 4 of this series.

The tables show quite clearly that no matter which value is assumed as the true protein metabolism, in the first hours of the observation a large part of the specific dynamic action is at the expense of the oxidation of carbohydrates. This is true of all cases except that of Louis M., in whom the 100 gm. of fat taken with the meat probably affected the relationship. This finding is not in accord with the results of Gigon.<sup>28</sup> He used casein and found that the specific dynamic action was due to protein metabolism alone, and that carbohydrate and fat were even spared slightly. Gigon's results on the increased heat production fol-

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26. Rubner, Max: *Die Gesetze des Energieverbrauchs bei der Ernährung*, Leipzig, 1902, p. 369.

27. Wolf, C. G. L.: *Die Ausscheidungszeit von Stickstoff, Schwefel und Kohlenstoff nach Aufnahme von Eiweisssubstanzen und Spaltungsprodukten*, Biochem. Ztschr., 1912, **40**, 234; *ibid.*, 1912, **41**, 111.

28. Gigon, A.: *Ueber den Einfluss der Nahrungsaufnahme auf den Gasaustausch*, Arch. f. Physiol., 1911, **140**, 509.



lowing casein ingestion are of interest in relation to the percentage increase which we have found. The results obtained by Gigon were recalculated by Williams, Riche and Lusk,<sup>20</sup> and the chart of their findings is here reproduced (Table 5). It shows an average specific dynamic action (70) which is lower than that reported in this paper (76). This difference is possibly due to the different form of protein ingested.

Staehelin<sup>29</sup> obtained, in one observation, results which are quite different from those of Gigon or from those here reported. He used a Jaquet apparatus, determining the metabolism in two hourly periods. The test meal consisted of 75 gm. of protein and 12.2 gm. of fat. In the twelve-hour observation following the meal the urine nitrogen increased 0.37 gm. per hour, which represents 9.9 calories of increased protein metabolism. The respiratory observation, however, indicates

TABLE 5.—INCREASE OF METABOLISM IN MAN DUE TO PROTEIN INGESTION (GIGON)

Food Casein, Gm.	Increase in Protein Metabolism		Increase in Total Calories of Metabolism	100 Calories of Protein Metabolism Increases Total Calories by
	Gm.	Calories		
50	7.1	28.4	19	67
100	23.8	95.2	53	56
150	35.7	142.8	118	83
200	58.1	232.4	171	74
Average.....				70

an average increased elimination by the body of 24.6 calories per hour and an increase in the proportion of fat metabolized. This result is not in accord with the other experiments here recorded.

Another conclusion may be drawn from the observations as viewed in Table 6. The relationship between the normal and abnormal cases shows that the specific dynamic action did not follow the surface area. The three normal cases have values per square meter which are very close together. The percentage increase of the two abnormal cases, however, is distinctly higher, which shows that diminishing the surface area or muscle volume did not proportionally reduce the intensity of the specific dynamic action. In the last column of the chart is shown the total percentage increase, found by dividing the actual increase in metabolism by the calories of extra protein oxidized. If one allows for the increased heat due to the fat ingestion of Louis M., the ratios

29. Staehelin, R.: Versuche ueber Gaswechsel und Energieverbrauch nach Nahrungsaufnahme, *Ztschr. f. klin. Med.*, 1908, **66**, 201.

are all closely related. We interpret this to mean that the specific dynamic action is dependent in these cases on the total protein metabolism. It is certainly independent of the mass of muscle tissue.

#### NORMAL CONTROLS

In the course of these experiments and of those recorded in Paper 20 of this series there have been six basal observations on normal men between the ages of 23 and 34. Four are new subjects whose metabolism has not been previously determined.

The basal metabolism of E. F. D. B. was determined four times in the spring of 1913, when an average value of 39.8 calories per square meter per hour was found. In May, 1915, the metabolism by the same index was 37.6 calories. In May, 1916, the height of his metabolism was 38.4 calories. This shows the constancy of the basal metabolism of some normal individuals.

TABLE 6.—SPECIFIC DYNAMIC ACTION. PERCENTAGE INCREASE

Name	Physical Condition	Urinary Nitrogen		Calories Extra Protein Metabolized per Hr. Extra N $\times 26.5$	Actual Calories Increase per Sq. M. per Hr.	100 Calories of Protein Metabolism Increases Cals. per Sq. M. by	Actual Total Calories Increase per Hr.	100 Calories of Protein Metabolism increases Total Calories by
		Basal, per Hr.	After Meat, per Hr.					
R. DeP.	Achondroplasia	0.60	1.88	33.9	19.4	57.2	24.1	71.1
Harry J.	Legless man	0.21	1.30	28.9	16.6	57.5	22.3	77.2
Louis M.*	Normal control	0.52	1.14	16.4	8.3	50.5	15.3	93.1*
W. B.	Normal control	0.38	1.22	22.3	11.7	52.6	18.2	81.8
S. K.	Normal control	0.46	1.29	22.0	11.1	50.5	16.6	75.5

\* Regular protein meal plus 100 gm. of fat.

The metabolism of J. H. M. had been determined by the Benedict unit apparatus and reported in the series of Palmer, Means and Gamble,<sup>30</sup> and of Means.<sup>2</sup> The average of these observations is 39.4 calories per square meter per hour. These findings were made more than a year before our determination (39.5 calories) and on a different type of apparatus.

The average calories per square meter per hour of these six controls is 38.9. The average found in this laboratory for normal men between the ages of 20 and 50 is 39.7 calories per square meter per hour. The six controls reported here average 2 per cent. below this figure (Table 7).

30. Palmer, W. W., Means, J. H., and Gamble, J. L.: Basal Metabolism and Creatinin Elimination, Jour. Biol Chem., 1914, **19**, 239.

TABLE 7.—SUMMARY OF BASAL METABOLISM OF SIX NORMAL CONTROLS. MEN

Name	Date	Age	Height, Cm.	Weight	Surface Area, Sq. M. Linear Formula	Calories per Sq. M. per Hour	Variations from Average Normal 39.7 %	Average R. Q.	Total Calories Measured		Divergence of Direct from Indirect, per Cent.
									Indirect	Direct	
E. F. D. B. ....	4/12/16	34	179.5	76.47	1.94	38.8	-2.2	0.80	150.71	161.79	+7.4
	4/25/16*	..	....	....	1.95	37.6	-5.4	0.80	73.23	71.54	-2.3
J. H. M. ....	4/21/16	30	177.0	75.34	1.89	39.5	-0.6	0.80	149.12	155.04	+4.0
J. C. F. ....	4/27/16	23	178.0	64.29	1.75	41.7	+5.1	0.82	146.05	149.13	+2.2
J. C. A. ....	4/28/16	26	175.2	60.20	1.64	39.2	-1.3	0.86	128.46	149.49	+16.3
S. K. ....	5/16/16	33	161.5	49.53	1.50	36.8	-7.4	0.79	110.31	114.84	+4.1
G. T. B. ....	5/23/16	25	164.5	54.37	1.56	38.7	-2.4	0.83	120.87	127.17	+5.2
Average.....	.....	..	....	....	....	38.7	-2.4	....	.....	.....	+5.7

\* One hour experiment. All others two hours.



## SUMMARY

1. The basal metabolism of five dwarfs, two legless men and six normal controls is reported. The legless men and the dwarfs, with apparently normal endocrine systems, showed, in relation to their surface area, the same level of metabolism as normal men. The law of surface area holds good for men of unusual body shape.

2. The dwarfs with involvement of the ductless glands and symptoms of cretinism showed a marked reduction in metabolism below the average found in normal cases, as has been reported by other authorities.

3. Following the ingestion of large quantities of meat, the excretion of urinary nitrogen during the earlier hours is not an accurate index of the protein metabolism. The sulphur excretion is more rapid than the nitrogen excretion.

4. The stimulation of metabolism following a large amount of meat is almost at its height two hours after the meal is eaten. The extra heat produced may amount to three-quarters of the calories in the protein metabolized, and may lead to an increase of 46 per cent. above the level of the basal heat production.

5. The specific dynamic action of a meal containing 24 gm. of nitrogen in the form of meat was larger in the case of a legless man, and of an achondroplastic dwarf, with very small arms and legs and normal trunk, than in the cases of three normal controls of greater weight and greater surface area. This indicates that the intensity of the specific dynamic action is not proportional to the mass of the musculature. The true explanation of the results cannot be given in the light of present knowledge. Various possible explanations come naturally to mind, such, for example, as a greater concentration of amino-acids in the blood flowing to the muscles, or the presence of a liver, which, in proportion to the size of the organism, is relatively larger than the normal.

477 First Avenue.

# CLINICAL CALORIMETRY

TWENTY-SECOND PAPER

## THE RESPIRATORY METABOLISM IN NEPHRITIS\*

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WITH THE TECHNICAL ASSISTANCE OF G. F. SODERSTROM

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A study of the syndrome known as *nephritis* should throw a great deal of light on many problems of normal and pathologic physiology. The disease is accompanied by profound changes of long duration, and the organism is obliged to adapt itself to conditions which are very different from those found in health. For this reason the investigator can obtain in the study of nephritis much information as to the manner in which the total metabolism is influenced by certain factors. Chief among these are nitrogen retention, edema, high blood pressure, acidosis and the conditions which cause uremia. Unfortunately, we know little about these factors, and suspect the presence of many more about which we know nothing. At this stage, all that one can do is to present his findings as completely as possible in the hope that they will be of service to those who can some day solve the nephritis problem. In the meantime, the study will help in the solution of many related questions.

The basal metabolism of patients with cardiac and renal disease was discussed briefly in Paper 16 of this series. The literature was reviewed and the results obtained on sixteen patients were published in such detail as was possible. Some of these patients had been studied in the early days of the calorimeter, before the general recognition of the importance of many of the modern measurements and tests. In most of the patients studied the cardiac element predominated, but in about half there was an important nephritic element also. It was found that the total metabolism of patients with mild cardiac or renal disease was normal, and that five of the twelve patients with dyspnea showed an increase in metabolism of 25 to 50 per cent. The respiratory quotients were all within normal limits and the method of direct calorimetry gave results only 1.9 per cent. lower than the method of indirect calorimetry. No direct relationship was found between the level of the metabolism and the acidosis, height of blood pressure or the ability of the kidney to excrete phenolsulphonephthalein. In

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\* From the Russell Sage Institute of Pathology, in affiliation with the Second Medical Division, Bellevue Hospital.

Table 2 of Paper 16 the results of the clinical and laboratory findings were compared.

This present work is a continuation of Paper 16 and Tables 2 and 3 have been made to correspond as closely as possible with Table 2 of the previous publication. More attention has been given to blood analyses, the methods for estimating the degree of acidosis, and the functional capacity of the kidneys. The patients were all primarily nephritics and were selected from a large number in the effort to obtain young people with few complications. For the first time since the calorimeter work was begun, patients with an absolutely bad prognosis were used as subjects.

#### METHODS

The men patients were studied in the metabolism ward and calorimeter fully described in previous papers. The two women patients could not be brought to the metabolism ward, and it was impossible to study their intake and output, but otherwise they were treated in just the same manner as the men. Patients with orthopnea were placed in the steamer chair while in the calorimeter; all others used the bed, with head rest and pillow. Most of the patients were given a cup of "caffeine-free" coffee (Kaffee Hag) without cream or sugar, early in the morning of the experiment. Except for this they were all fasting, having received no food since the previous afternoon. While in the apparatus they were all quiet, except the uremic patients, Isadore R. and Frank C.

The blood pressure was measured by the Faught apparatus, diastolic readings being made at the diminution of the murmur in the artery, which usually occurs just before its disappearance. The phenolsulphonephthalein test of Rowntree and Geraghty was used, the results in the tables being expressed as the total excretion in two hours and ten minutes. The carbon dioxide combining capacity of the plasma was determined by the Van Slyke method.

The blood and urinalyses, while the patients were in the metabolism ward, were made by Dr. Frank C. Gephart of the staff of the Russell Sage Institute of Pathology. While the patients were in the general medical wards the analyses were made, for the most part, in the Pathological Department of Bellevue Hospital by Drs. A. O. Gettler and Willis Baker. To them and to Dr. Norris we are indebted for permission to publish the results. These authors have recently published a valuable contribution on the "Chemical and Physical Analysis of Blood in Thirty Normal Cases."<sup>1</sup>

#### DISCUSSION OF RESULTS

The manifestations of nephritis are so varied that it will require the study of many cases to give all the information that is needed. Nevertheless, much can be learned from the ten cases summarized in Table 2 and the sixteen cases of the previous publication. (See Table 2, Paper 16).

*Direct and Indirect Calorimetry.*—In the thirteen<sup>2</sup> experiments here reported the total calories, as measured by the method of indirect

1. Gettler, A. O., and Baker, Willis: Jour. Biol. Chem., 1916, **25**, 211.

2. The experiment on Mildred C. is omitted because the direct method could not be used.



calorimetry, was 1,700.4; by the method of direct calorimetry, 1,743.3, or 2.5 per cent. higher. In the previous work, the calories by the indirect method totaled 4,297.7, and by the direct method, 4,214.5, which was 1.9 per cent. lower. These two divergences of the direct calorimetry almost neutralize each other, and in the grand total of all the work the indirect is 5,998.1; the direct, 5,957.8, which is 0.7 per cent. lower. This agreement is just as close as in the case of a group of normal controls, and it shows that the calorific value of one liter of oxygen is the same in nephritics and normals. We must not forget, however, that there may be striking changes in the intermediary metabolism without great change in the calorific value of oxygen. This is brought out clearly in the work on diabetes.

*Respiratory Quotients.*—Except in the cases of diabetes and in convalescence, the respiratory quotient fourteen hours after the last meal is little more than the expression of the amount of carbohydrate available in the organism. This depends on the state of nourishment, the amount of carbohydrate food taken the preceding day, and the level of the total metabolism which determines the rate at which this carbohydrate is oxidized. Nephritic patients, as a rule, are given carbohydrate diets unless the total calories are greatly restricted. It is not surprising, therefore, that quotients as high as 0.93 and 0.89 were found. On the other hand, the lowest quotients obtained, 0.77 and 0.78, are well within normal limits. This confirms the statement made a year ago that there is no need to assume any change in the type of the metabolism from the normal.

*Total Metabolism.*—The calculations of the total metabolism are based on the measurements obtained by the method of indirect calorimetry and are expressed as calories per square meter of surface per hour. This figure is then compared with the standard average normal figure for the same age and sex, as given in Table 7 of Paper 13 of this series, and the percentage variation from the normal recorded. Most of the subjects could be compared with the group of normal men between the ages of 20 and 50, the average calories per square meter per hour being 39.7. The results obtained on John C., 62 years old, were compared with the figure 37, which is about the average for his time of life according to the revised curve given in Paper 19. This makes him 10 per cent. above the normal. He still had his youthful vigor, and it is well to remember that his metabolism was only 3 per cent. above the standard for men 20 to 50 years of age. Edna S. presents a different problem. She was only 13 years old, and the standard for girls of this age is 47 calories per square meter per hour. For two months before the calorimeter observation she had been on a restricted

diet, and this may reduce the metabolism even more in a child than in an adult. Direct evidence on this point is lacking, but it can be seen in Table 9 of Paper 17 that the four children with severe diabetes show an unusual reduction in metabolism. The observation on Frank C., with uremia, was almost spoiled by his restlessness, which was even greater than is indicated by the high work-adder. The results were 29 per cent. above the basal standard, but those who observed him were confident that his muscular activity alone could have increased his metabolism 20 to 30 per cent., and it is doubtful if his basal metabolism would have been higher than the average normal. It is unfortunate that the otherwise satisfactory experiment on Mildred C. should be marred by the possibility that she had exophthalmic goiter. Her nervous temperament and exophthalmos, which was greater than is generally found in nephritis, suggested this factor.

*A. Influence of Dyspnea.*—Isidore R., Frank C., and John C. were distinctly dyspneic, and they all showed a slight increase in metabolism. This increase was not as marked as in the four out of five patients with moderately severe dyspnea described in the paper on cardiacs and nephritics.

*B. Acidosis.*—According to the carbon dioxid combining capacity of the plasma, a rather severe type of acidosis was present in the case of Joseph U. on April 5, and in Isidore R. on January 24. The former showed a metabolism of 7 per cent. below the normal; the latter, only 2 per cent. above. Slight acidosis was found in Joseph U. on April 10, and in Jack K. on March 4. Their figures were  $-12$  per cent. and  $+5$  per cent., respectively. These results, taken with the figures obtained in Papers 16 and 17, indicate that acidosis has little effect on the total metabolism.

*C. Edema.*—There was marked edema in the cases of Joseph U., Lee H., Edna S. and William S. The first three showed a distinct reduction in metabolism. With Adam P., January 14, the edema was moderate and the metabolism 10 per cent. below the average normal. In general, the type of nephritis with edema is accompanied by a reduction in metabolism; the type without edema, by a slight increase in metabolism. One naturally expects an organism diluted by inert fluid to show a diminished metabolism according to body weight. Obesity patients have such a reduction, but Means has proved that they have the same metabolism per square meter of surface as normal people.<sup>3</sup>

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3. Means, J. H.: Studies of the Basal Metabolism in Obesity and Pituitary Disease, *Jour. Med. Research*, 1915, **32**, 121; The Basal Metabolism in Obesity, *THE ARCHIVES INT. MED.*, 1916, **17**, 704.

Edematous cardiac patients as a rule show an increased metabolism, but here other factors may be involved. The reduction of metabolism per square meter of surface found in nephritic edema is sometimes very marked ( $-27$  and  $-40$  per cent.), and it points to some cause other than mere dilution of tissue and distention of skin.

*D. High Blood Pressure.*—All the patients except Lee H. showed a rise in blood pressure. The systolic pressure was above 200 mm. in the cases of Adam P., January 14, Isidore R., Frank C., John C. and Mildred C. Of these, Adam P. showed a reduced metabolism and the others an increase. With Adam P. the metabolism was about the same when his systolic pressure was 185 mm. as when it was 230 mm. George M., of Paper 16, showed exactly the same figures for a blood pressure of 160 mm. as for a pressure of 195 mm. On the other hand, Joseph U. showed a drop in metabolism when his blood pressure fell. Blood pressure has no constant influence on the total metabolism.

*E. Eliminative Power of the Kidney.*—Curiously enough, most of the patients were able to eliminate at least 6 gm. of nitrogen in the urine on the calorimeter days, and Lee H. was the only one, except the incontinent patients, Isadore R. and Frank C., in whom the urine volume was much diminished. On the other hand, the chlorid elimination was low in most of the patients, probably on account of the low intake in some cases, but in most on account of inability on the part of the kidney to excrete salt. The phthalein output was low in all cases except Joseph U. on April 5, and Lee H. on December 4. The Ambard coefficient expressed in terms of the McLean index was low in the cases of Joseph U. and Frank C.

The nonprotein nitrogen was above the normal limits in the cases of Joseph U., Jack K., Isidore R., Frank C.; and the urea nitrogen was above normal in Lee H. also. One of these patients showed a decrease in metabolism, the others a slight increase. In general, it does not seem that the products retained by damaged kidneys have any direct effect on the level of the total metabolism.

#### CHANGES IN INDIVIDUAL CASES

Joseph U. was in the calorimeter three times, and during the whole period there was little change in the McLean index and blood urea. In the first experiment his metabolism was higher than normal. In the next week he lost 5 kg. of edema and his metabolism fell to 7 per cent. below the normal average. At this time he had a distinct acidosis and furnished an excellent opportunity to study the effect of this factor on metabolism. Accordingly, he was given sodium bicarbonate until the acidosis almost disappeared and there was scarcely any change in his metabolism.



Lee H. was studied, December 4, and again a month later, when he had gained 5.5 kg. of edema. During this same period there was a marked fall in the phthalein output and increase in the retention of urea and creatinin. His metabolism in the first test had been 12 per cent. below the average normal; a month later it was 27 per cent. below. Part of this decrease was undoubtedly due to rest and low diet.

Adam P. was in the calorimeter January 14 and again three and a half weeks later, when he had lost 8 kg. of edema. In this interval there was practically no change in the phthalein excretion or in the blood urea, but the blood pressure fell 45 mm. There was hardly any alteration in the level of his total metabolism.

#### SUMMARY AND CONCLUSIONS

Ten patients with nephritis were studied in the calorimeter. All were severe cases, and it was possible to examine five of the patients shortly before death. The results have been compared with those obtained last year on a series of patients with cardiorenal disease, six of whom were primarily nephritics.

In the present series nine experiments were made on edematous subjects. All but two of these tests showed a diminution of the basal metabolism, both according to body weight and to surface area. In two cases with great edema the heat production was 27 and 40 per cent. below the normal average.

Five patients suffering from nephritis of the chronic interstitial type, without edema, were studied. One with marked uremia was at the normal level of metabolism. Another, who was very restless, showed an increase. A third, in whom hyperthyroidism was suspected, also showed an increase. The other two were near the upper normal level of metabolism.

In most of the patients with greatly increased blood pressure the metabolism was higher than in the other nephritics with lower blood pressures. Most of the patients with marked dyspnea showed some increase in metabolism. No relationship could be established between the level of metabolism and the degree of acidosis or the eliminative power of the kidney, as estimated by the McLean index, the phenol-sulphonphthalein test, the elimination of salt and nitrogen, and the analysis of the various substances in the blood, such as chlorids, urea, and the nonprotein nitrogen.

The methods of direct and indirect calorimetry in this and the previous series of cardionephritics give totals which agree within 0.7 per cent. The respiratory quotients are all within normal limits, showing that nephritics derive their energy from very much the same proportions of the various foodstuffs as do normal men.

The normal quotients found in patients with low carbon dioxid com-

binning capacity of the plasma prove that nephritic acidosis is not caused by difficulty in oxidizing carbohydrates.

Edematous nephritics kept on low diets show a reduction in food requirement similar to that usually found in prolonged undernutrition. Other nephritics have approximately the normal food requirement.<sup>4</sup>

#### CASE HISTORIES

CASE 1.—Joseph U., aged 19; chronic parenchymatous nephritis with acute exacerbation. Admitted March 27, 1916; died April 23, 1916.

*History.*—Five years prior to the test he had scarlet fever. Recently he has been a sign painter's helper but has not been much exposed to the danger of lead poisoning. His habits are good. For several years he has passed his water once or twice during the night. Early in March, 1916, after a wetting, he noticed edema of the eyelids, then edema of the ankles, disappearing at night. About March 20 the edema of the legs became permanent and a few days before admission the genitalia became edematous. He feels perfectly well.

*Physical Examination.*—Height 175 cm.; well nourished; color pasty; marked edema of face, back, genitalia, thighs and legs. Heart: Apex beat in the fifth space 11.5 cm. to the left of the median line. Left limit of dullness 12.5 cm. to the left; right limit, 3 cm. to the right of median line. Sounds are of good quality; no murmurs. Lungs are clear. There is marked acne of face and back.

The clinical data are given in Table 4 and a summary of the more important findings in Tables 2 and 3. His urine contained a large amount of albumin, pus casts and many granular casts; no glucose. The McLean indexes were as follows: March 31, 49; April 4, 46; April 10, 39. The CO<sub>2</sub> combining power of the plasma was as follows: April 4, 39 volume per cent.; April 5, 39 per cent.; April 8, 40 to 50 per cent.; April 9, 60 per cent.; April 10, 55 per cent.; April 16, 39 per cent.; April 19, 48 per cent.

Calorimeter observations were made on March 29, April 5 and April 10.

From March 29 to April 10 he was on a mixed diet containing about 1,500 calories, 5 gm. nitrogen and 1.5 gm. salt, with about 1,500 c.c. fluids in addition to the water of the so-called solid foods. He was given hot air baths almost every day, responding unusually well, losing between 480 and 900 gm. weight during the baths. His temperature was normal, he felt well and he lost 5 kg. weight in twelve days, the edema almost disappearing. During this period he passed 1,000 to 1,500 c.c. urine a day, containing from 10 to 13 gm. of nitrogen and 2 to 5 gm. of chlorids. The prognosis would have been favorable were it not for the low phthalein output on two occasions, the low McLean index, the high nonprotein nitrogen and high urea of the blood and the low carbon dioxid combining power of the plasma. The high phthalein output of April 4 stood in contrast to the other tests.

In order to eliminate the factor of acidosis he was given 40 gm. sodium bicarbonate between 6 p. m., April 8 and 6 p. m., April 9, the two days before the last calorimeter test.

On April 11 the picture changed markedly. He became very weak in the hot air bath and was removed at once. The temperature rose to 105 F. and remained elevated for five days. During this period his pulse was 98 to 124; respiration, 24 to 40. The edema increased. On the day after the onset,

4. The above work is a continuation of studies in cardiac and renal disease carried on a year previously in association with Dr. Francis W. Peabody of Boston. It is a pleasure to record the active participation of Dr. Peabody in about half of the experiments of this present series.

TABLE 1.—NEPHRITIS. DATA OF—

[illegible]



873

—CALORIMETER EXPERIMENTS

Direct Calorimetry, Cal.	Rectal Temp., C.	Average Pulse	Work-Adder, Cm.	Non-protein R. Q.	Per Cent. Calories from			Calories per Hour		Remarks
					Protein	Fat	Carbo-hyd.	Per Kg.	Per Sq. M. (Lin.)	
.....	37.1	....	.....	.....	..	..	..	....	....	Basal; in bed
84.4	37.1	66	19.0	0.76	..	..	..	....	....	Quiet; reading 15 minutes
81.2	37.0	66	19.0	0.77	..	..	..	....	....	Quiet; not reading
.....	.....	.....	.....	.....	18	66	16	1.10	45.1	
.....	36.9	.....	.....	.....	..	..	..	....	....	Basal; in bed
66.2	36.7	55	16.0	0.72	..	..	..	....	....	Quiet
79.6	36.7	55	5.0	0.88	..	..	..	....	....	Very quiet
73.6	36.7	55	15.5	0.92	..	..	..	....	....	Very quiet
.....	.....	.....	.....	.....	15	66	19	0.95	36.9	
.....	36.9	.....	.....	.....	..	..	..	....	....	Basal; in bed
74.0	36.9	63	12.0	0.81	..	..	..	....	....	Quiet; asleep 15 minutes
85.0	37.2	64	4.0	0.77	..	..	..	....	....	Motionless
.....	.....	.....	.....	.....	16	61	23	0.99	38.0	
.....	36.6	48	.....	.....	..	..	..	....	....	Basal; in chair
48.4	36.5	44	15.0	0.84	..	..	..	....	....	Fairly quiet; dozed
54.1	36.4	44	15.0	0.84	..	..	..	....	....	Fairly quiet
.....	.....	.....	.....	.....	12	44	44	0.98	34.5	1st period—57½ min.
.....	36.8	61	.....	.....	..	..	..	....	....	2d period—62½ min.
54.9	36.9	67	10.0	0.86	17	41	42	....	....	Basal; in bed
52.4	36.8	68	14.0	0.79	15	62	23	....	....	Very quiet
.....	.....	.....	.....	.....	..	..	..	0.80	29.0	Very quiet, until end of period; 61 minute period
.....	37.0	.....	.....	.....	..	..	..	....	....	Basal; in chair
37.3	37.0	82	1.0	0.94	..	..	..	....	....	Very quiet; reading
40.7	37.0	83	2.4	0.90	..	..	..	....	....	Very quiet
21.8	37.0	80	1.5	0.97	..	..	..	....	....	Very quiet; reading; 30 minute period
.....	.....	.....	.....	.....	5	21	74	0.81	28	
.....	37.2	.....	.....	.....	.....	.....	.....	.....	.....	
55.9	37.1	58	6.0	0.82	16	52	32	....	....	Very quiet
58.0	37.0	59	25.0	0.78	16	63	21	....	....	Somewhat restless
.....	.....	.....	.....	.....	..	..	..	0.95	35.9	
.....	36.9	.....	.....	.....	..	..	..	....	....	Basal; in bed
53.3	36.9	60	13.0	0.77	8	71	21	....	....	Very quiet
49.6	36.8	53	15.0	0.84	9	50	41	....	....	Quiet
.....	.....	.....	.....	.....	..	..	..	1.00	34.4	
.....	37.1	.....	.....	.....	..	..	..	....	....	"Basal"; in bed; irrational
41.0	37.0	92	23.0	.....	..	..	..	....	....	Fairly restless; 30 min. period
44.3	37.0	96	30.0	.....	..	..	..	....	....	Restless; 30 min. period
38.2	37.1	.....	6.0	.....	..	..	..	....	....	Asleep; very quiet; 30 min. period
.....	.....	.....	.....	.....	..	..	..	1.24	41.9*	

\* Results closer to true basal figures are obtained by using average R. Q. and taking CO<sub>2</sub> figures for first and third periods. This gives an average of 40.4 calories per square meter per hour.

TABLE 1.—NEPHRITIS. DATA OF—

Subject, Date, Weight, Surface Area, Linear Formula	Period	End of Period	Carbon Dioxid, Gm.	Oxygen, Gm.	R. Q.	Water, Gm.	Urine N per Hour, Gm.	Indirect Calo- rimetry, Cal.	Heat Elimi- nated, Cal.
Case 5 (Jack K.)... 3/4/16 58.3 Kg. 1.62 Sq. M.	Prelim.	11:58	.....	.....	.....	.....	.....	.....	.....
	1	12:58	24.7	20.1	0.89	23.3	0.525	68.2	69.0
	2	1:58	23.9	19.6	0.89	25.1	0.525	66.3	75.1
	Aver.	.....	.....	.....	.....	.....	.....	.....	.....
Case 6 (Frank C.) 2/28/16 Missing 1.54 Sq. M.	Prelim.	11:46	.....	.....	.....	.....	.....	.....	.....
	1	12:44	23.2	23.4	0.72	21.1	0.525	.....	67.4
	2	1:21	18.1	14.2	0.92	16.0	0.525	.....	47.8
	Total 95 min. Aver. per hr. Prelim.	.....	.....	.....	.....	.....	.....	124.8	.....
Case 7 (John C.)... 2/9/16 69.8 Kg. 1.87 Sq. M.	Prelim.	.....	.....	.....	.....	.....	.....	.....	.....
	1	12:40	24.3	23.9	0.74	32.3	0.276	78.4	81.7
	2	1:40	25.0	22.1	0.82	30.9	0.276	74.3	79.9
	Aver.	.....	.....	.....	.....	.....	.....	.....	.....
Case 8..... (Mildred C.) 12/10/16 49.7 Kg. 1.40 Sq. M.	Prelim.	11:57	.....	.....	.....	.....	.....	.....	.....
	1	12:57	19.9	16.6	0.87	21.6	0.231	.....	49.7
	2	1:57	21.4	23.1	0.68	31.6	0.231	.....	61.9
	Aver.	.....	.....	.....	.....	.....	.....	65.8	.....
Case 9 (Wm. S.)... 12/6/16 64.2 Kg. 1.66 Sq. M.	Prelim.	11:40	.....	.....	.....	.....	.....	.....	.....
	1	12:40	22.2	20.9	0.77	26.1	0.483	68.8	68.3
	2	1:40	22.4	20.6	0.79	27.5	0.483	68.3	70.5
	Aver.	.....	.....	.....	.....	.....	.....	.....	.....

bronchial voice and breathing were heard over the right lower lobe. Two days later there was a small area of dulness and bronchial breathing at the angle of the left scapula. April 16 the temperature was normal and the patient felt weak but much better. By the 19th he was drowsy and his respirations slow and deep. There was persistent flatness and diminished breathing over the right lower lobe, but only 2 c.c. cloudy, sterile fluid could be withdrawn by needle. On the afternoon of the 22d he suddenly began to have violent convulsions, lasting until his death, shortly after midnight. No necropsy could be obtained.

After the febrile attack, which was probably pneumonia, the urine volume diminished rapidly, with a marked fall in the output of nitrogen and salt. This was in part due to a diarrhea.

CASE 2.—Lee H., aged 21, Chinaman, cook; chronic parenchymatous nephritis with acute exacerbation. Admitted Nov. 19, 1915, died Jan. 14, 1916.

*History.*—It was impossible to get any information as to the character of his previous illnesses. He says that he has always been well. He has been in New York five years. He says his present trouble began about Nov. 1, 1915.

*Physical Examination.*—Height 170 cm.; frame small, whole body, including face and scalp, markedly edematous. His color is pasty; he is orthopneic and dyspneic. Heart: Apex beat in the fifth space 10 cm. from median line; no murmurs. Lungs: At both bases, flatness, diminished breath sounds and

## —CALORIMETER EXPERIMENTS—(Continued)

Direct Calorimetry, Cal.	Rectal Temp., C.	Average Pulse	Work-Adder, Cm.	Non-protein R. Q.	Per Cent. Calories from			Calories per Hour		Remarks
					Protein	Fat	Carbohyd.	Per Kg.	Per Sq. M. (Lin.)	
.....	36.9	72			..	..	..	....	....	Very quiet; basal
63.4	36.8	60	5.0	0.92	..	..	..	....	....	Asleep 35 min. Reading 10 min.
68.0	36.7	68	13.5	0.92	..	..	..	....	....	Very quiet; awake
.....	.....	.....	.....	.....	21	23	56	1.2	41.5	
.....	37.0	88	.....	.....	..	..	..	....	....	Basal (restless)
71.0	37.1	86	47.0	.....	..	..	..	....	....	58 min. period; very restless
50.6	37.2	89	54.0	.....	..	..	..	....	....	37 min. period; very restless
76.8	.....	.....	.....	0.80	18	57	25	1.6	51.2	Very unsatisfactory observation
.....	37.2	68								
74.9	37.1	57	19.0	0.74	..	..	..	....	....	Very quiet
75.5	37.1	60	18.5	0.83	..	..	..	....	....	Quiet
.....	.....	.....	.....	0.78	10	68	22	1.1	40.8	
.....	.....	104	.....	.....	..	..	..	....	....	Basal; in chair
.....	.....	108	5.0	0.88	..	..	..	....	....	Very quiet; slept
.....	.....	104	11.0	0.66						
.....	.....	.....	.....	0.77	9	73	18	1.32	47.0	Rectal thermometer out of order
.....	38.1	85								
74.9	38.3	86	6.0	0.76	..	..	..	....	....	Quiet
71.1	38.3	88	5.0	0.79	..	..	..	....	....	Quiet
.....	.....	.....	.....	.....	19	62	19	1.07	41.2	

fremitus. Radial arteries slightly thickened. Eyegrounds normal. Abdomen contains some fluid.

Patient was in the calorimeter December 4 and January 5. For clinical data December 4 and January 5 to 12, see Table 6, and also the summary, Tables 2 and 3.

The urine contained a large amount of albumin and many hyaline and granular casts. The specific gravity was high, usually in the neighborhood of 1.040. Glucose was absent. On some examinations red blood cells were found in the urine. The Wassermann test was faintly positive on three examinations. November 24 the blood urea was 32 mg.; creatinin, 0.84 mg.; chlorids, 0.51 mg.; freezing point,  $-0.647^{\circ}\text{C}$ .; December 4, alveolar  $\text{CO}_2$  38 mm. Hg; January 4, blood urea was 49 mg.; ammonia nitrogen, 1.1 mg.; chlorids, 0.612 per cent.; creatinin, 3.9 mg.;  $\text{CO}_2$  combining capacity, 70 per cent. The phenolsulphone-phthalein excretion in two hours was as follows: December 3, 64 per cent.; December 18, 44 per cent.; January 12, 13 per cent.

The blood pressure was not increased: Systolic, 115 to 120 mm.; diastolic, 75 to 85 mm.

The patient was kept on a moderately restricted diet and was given hot packs. His edema continued to increase but the dyspnea and orthopnea were not distressing and the patient remained cheerful. The physical signs of fluid in the pleural cavities and abdomen were practically unchanged.



January 4 he began to have fever in the afternoons, reaching 101 to 103 F., with morning remissions. He complained of pain in the abdomen and chest and began to cough. Over the lungs, anteriorly, there developed many moist, crackling râles, and over the precordium a cardiorespiratory friction rub. The urine volume fell to 600 to 900 c.c., with 11 to 17 gm. nitrogen and only a trace of chlorids. His bowels moved four to seven times a day. The patient grew more toxic and dyspneic but had no convulsions. He died, January 13. Postmortem aspiration of the pleural cavities and abdomen yielded milky fluid containing very few cells, but many diplococci. There was no necropsy.

The blood on January 7 showed hemoglobin, 75 per cent.; red blood cells, 4,500,000; leukocytes, 10,000; no filaria.

CASE 3.—Edna S., aged 13, schoolgirl, born in New York. Admitted Oct. 7, 1915; died May 2, 1916; chronic parenchymatous nephritis with acute exacerbation.

*History.*—Previous history negative except for measles and a mild sore throat. In 1912 she had a cough for a month and went to a physician who said she had kidney trouble. For the previous two years she had had frequent nosebleeds, but no frequency of micturition, headache, dizziness or edema until the present trouble. Sept. 1, 1915, her mother noticed edema of the face. October 4 she was put to bed by the family physician on account of edema of the legs. October 6 she lost her vision and had a convulsion of the right side of the body, and was also in a convulsion when she was brought to the hospital the next day.

*Physical Examination.*—Color pasty; face, body and extremities very edematous. The patient is a well nourished, intelligent girl, orthopneic and slightly dyspneic. Respirations, Cheyne-Stokes. Tonsils large, with deep crypts. Heart: Left border of dullness in the fifth space 2 cm. to the left of the mid-clavicular line; right border 2 cm. to the right of the midsternal line. The second sound in the aortic area is markedly accentuated; no murmurs. The liver edge is felt 2 cm. below the free margin of the ribs. The fundi of the eyes show slight haziness of the disk outlines. The blood pressure, which was 160 mm., dropped to 115 mm. after removal of 350 c.c. blood from vein.

On repeated examinations the urine showed large amounts of albumin, many hyaline and granular casts, and sometimes red blood cells. It was acid, contained no glucose, and the specific gravity averaged about 1.020.

The patient was given a restricted diet, with fruit and plenty of carbohydrate, but very little nitrogen and only small amounts of fluid. She was also given a daily hot pack. November 15 and again March 3 she had convulsions during which venesections were performed. In November and December there was little change in her general condition, the edema being extreme and the urine volume always scanty. The patient was in the calorimeter December 1.

January 28 fluid began to collect in the left pleural cavity. April 18 the temperature, which had been normal, rose to 101 F., then became very irregular, reaching 106 F., April 29. Cough and pain in the chest developed. She became more and more toxic and stuporous and died May 2, 1916. No necropsy could be obtained.

The phthalein tests were as follows: November 15, 16 per cent.; November 23, 61 per cent.

The blood tests: November 15: urea nitrogen, 14.5 mg.; November 18: urea nitrogen, 22.6 mg.; November 30: nonprotein nitrogen, 18.9 mg.; March 3: total nonprotein nitrogen, 19.7 mg.; urea nitrogen, 16.2 mg.; blood CO<sub>2</sub> combining power, 62 volume per cent.

The spinal fluid, March 3, removed when the patient was stuporous, ten minutes before the convulsions began, was under increased pressure.

CASE 4.—Adam P., aged 26, Russian, a tailor. Admitted Jan. 10, 1915; discharged improved, May 5, 1916; chronic parenchymatous nephritis with acute exacerbation.

*History.*—His father was a heavy drinker. At the age of 15 the patient had rheumatic fever for two months, involving many joints. Since then he has had some subacute symptoms. Six months prior to the test he had tonsillitis. He uses beer in moderation.

About April, 1915, he noticed edema of the legs, which has gradually increased and has involved abdomen, arms and face. Shortly afterward, he began to suffer from dimness of vision, dizziness and headaches. He has had increasing nocturia and now passes urine three to four times at night.

*Physical Examination.*—A well developed young man with marked generalized edema. He is not dyspneic. Heart: Left border of dulness 12.5 cm. from the median line. The second pulmonic sound is accentuated. At the bases of the lungs there is some dulness and diminution of breath sounds. Eyes: Fundi show chorioretinitis of long duration. He can distinguish fingers only at a distance of 2 feet. Dr. Rees believes that the eye condition has nothing to do with the nephritis.

The urine contained a large amount of albumin and many hyaline and granular casts. The specific gravity varied between 1.016 and 1.025. On admission the blood analysis was as follows:

Sugar .....	90	mg.
Urea nitrogen .....	34.3	mg.
Ammonia nitrogen .....	0.25	mg.
Creatinin .....	0.5	mg.
Uric acid .....	3.7	mg.
Plasma chlorids .....	0.60	per cent.

The phthalein test in two hours was 27 per cent. and the McLean index was 67.

The blood pressure was about 200 mm. systolic and 130 mm. diastolic until February 3; then it fell to about 160-105 mm.

The patient was given a restricted diet and, at first, hot packs. The generalized edema and hydrothorax continued. He was in the calorimeter January 14. At this time there were about 10 kg. of retained fluid in the edema. The daily urine volume was 1,000 to 1,500 c.c.; urine nitrogen, 8 to 11 gm.; chlorids, 5 to 8 gm. The patient lost weight steadily and when next in the calorimeter, February 7, had almost no edema. The McLean index February 8, was 25. The output of water, nitrogen and chlorids in the urine exceeded the intake. The calories in the diet were just about sufficient to cover the requirement. The volume per cent. of CO<sub>2</sub> in the plasma was as follows: January 13, 58; January 18, 62; February 8, 66.

The patient continued to improve slowly and by March 5 had no edema and no dyspnea and was able to get out of bed for a few hours. He was still unable to distinguish fingers at a greater distance than 4 feet. March 5, after walking a few steps, he complained of pain in the back. The next day the urine contained blood. For the following week he had pain over the right kidney, slight fever, many pus cells and a few red blood cells in the urine. The Roentgen ray revealed no calculus. Infarction of the kidney was suspected.

The pus in the urine disappeared in a few weeks. On March 17 the urine culture showed streptococci. He grew stronger and on May 5 was sent to a convalescent home. While on measured diets several experiments were made to determine the effect of sodium bicarbonate on his weight and the carbon dioxid combining capacity of his plasma.

CASE 9.—William S., aged 27, laborer, was admitted to the Fourth Medical Division, service of Dr. Nammack, Nov. 16, 1915; discharged improved Feb. 4, 1916; chronic parenchymatous nephritis.

*History.*—At the age of 9 he had scarlet fever, followed by impairment of the hearing. Two years prior to the test he had urethritis. During the previous year the legs and ankles were swollen at intervals and the face and genitalia were at times edematous. Recently, he has had headaches and pain in the back and lower thoracic region. He has had no dyspnea or cyanosis.

TABLE 2.—NEPHRITIS. SUMMARY—

Case Number and Name	Date in Cal'r.	Age, Yrs.	Diagnosis	Status	Temp., C.
1. Joseph U. ....	3/29	19	Chronic parenchymatous, Ac. Exac. ....	Died 4/23	37.1
	4/ 5	..	.....	.....	36.7
	4/10	..	.....	.....	37.0
2. Lee H. ....	12/ 4	21	Chronic parenchymatous, Ac. Exac. ....	Died 1/14	36.5
	1/ 5	..	.....	.....	36.8
3. Edna S. ....	12/ 1	13	Chronic parenchymatous, Ac. Exac. ....	Died 5/2	37.0
4. Adam P. ....	1/14	26	Chronic parenchymatous, Ac. Exac. ....	Improved	37.1
	2/ 7	..	.....	.....	36.8
9. William S. ....	12/ 6	27	Chronic parenchymatous .....	Improved	38.3
5. Jack K. ....	4/ 4	28	Chronic interstitial .....	Improved	36.8
10. Isidore R. ....	1/24	26	Chronic interstitial, uremia.....	Died 1/27	37.1
6. Frank C. ....	2/28	19	Chronic interstitial, uremia.....	Died 3/27	37.1
7. John C. ....	2/ 9	61	Chronic interstitial .....	Improved	37.1
8. Mildred C. ....	12/10	89	Chronic interstitial (hyperthyroidism?)	Improved	....

TABLE 3.—NEPHRITIS. SUMMARY—

Case Number and Name	Date in Cal'r.	Urine				Ambard McLean Index	Blood	
		24 Hr. Volume, C.c.	Total N, Gm.	Total Cl as NaCl	Phthalein per Cent. in 2 Hrs.		Nonprotein N, Mg.	Urea, Mg.
1. Joseph U. ...	3/29	1,520	12.1	4.1	10	48	—	59.5
	4/ 5	946	9.1	1.8	79	47	100.0	57.4
	4/10	935	9.3	2.3	16	39	117.0	53.2
2. Lee H. ....	12/ 4	570	7.2	—	64	..	.....	32.0
	1/ 5	260	7.8	0.9	13	..	.....	49.0
3. Edna S. ....	12/ 1	.....	....	...	..	..	.....	....
4. Adam P. ....	1/14	1,280	7.7	4.8	27	67	39.4	20.5
	2/ 7	1,340	6.7	4.1	31	..	41.0	18.9
9. William S. ....	12/ 6	1,230	10.7	—	48	..	41.7	17.3
5. Jack K. ....	3/ 4	1,750	6.8	6.9	8	82	72.5	53.9
10. Isidore R. ....	1/24	.....	....	...	..	..	165.0	58.1
6. Frank C. ....	2/28	.....	....	...	18	49	49.7	18.9
7. John C. <sup>4</sup> .....	2/ 9	975	8.1	5.8	35	..	33.3	16.1
8. Mildred C. ....	12/10	.....	....	...	20	134	26.2	13.2

1. On November 24.

2. Below 47, the normal for a girl of this age.

3. Very restless.

4. 62 years old.



## —OF CLINICAL DATA

Pulse	Resp. Rate	Dyspnea	Orthopnea	Periodic Resp.	Cyanosis	Edema	Blood Pressure, Syst. Diast.	
66	.....	±	—	—	—	++	175	108
55	.....	—	—	—	—	+	144	84
64	.....	±	—	—	—	+	150	94
46	15-18	+	+	—	—	+	110	80
67	.....	+	+	—	—	++	115	85
82	.....	±	++	—	—	+++	148	105
59	.....	—	—	—	—	Mod.	230	140
57	.....	—	—	—	—	—	185	120
85	20-25	±	±	..	—	++	178	90
65	.....	—	—	—	—	—	197	145
94	.....	++	—	++	±	—	155	100
88	.....	+++	++	+	..	—	220	145
60	.....	+++	++	++	+	—	230	125
104	.....	+	+	..	—	—	260	170

## —OF LABORATORY DATA

Creatinin, Mg.	Blood		CO <sub>2</sub> Comb. Power, Volume per Cent.	Calories per Sq. M. per Hour	Variation from Average Normal Lin.	Calories per Kg.	R. Q.
	Total Chlorids, Mg.	CO <sub>2</sub> Comb. Power, Mm. Hg.					
....	0.649	..	....	45.1	+14	1.10	0.77
1.91	0.665	26	38.8	36.9	— 7	0.95	0.83
....	0.634	38	55.0	38.0	— 4	0.99	0.79
0.84 <sup>1</sup>	0.51 <sup>1</sup>	47	....	34.5	—13	1.00	0.84
3.9	0.612	..	70.2	29.0	—27	0.80	0.82
....	.....	..	....	28.0	—40 <sup>2</sup>	0.81	0.93
....	0.623	..	57.8	35.9	—10	0.95	0.80
....	0.672	..	66.3	34.4	—13	1.00	0.81
....	.....	31 <sup>5</sup>	....	41.2	+ 4	1.07	0.78
2.6	0.740	35	52.8	41.5	+ 5	1.15	0.89
....	0.609	19 <sup>6</sup>	43.6	40.4 <sup>7</sup>	+ 2	....	0.82 <sup>8</sup>
....	0.528	..	63.5	51.2 <sup>3</sup>	+29	1.56	0.82
....	0.594	..	69.1	40.8	+10	1.09	0.78
....	0.694	..	....	47.0	+27	1.32	0.77

5. Alveolar air.  
6. On January 21

7. Calculated from CO<sub>2</sub> of first and third periods.  
8. Somewhat restless throughout.

*Physical Examination.*—A somewhat undernourished young man with marked edema of the whole body, including face and genitalia. The heart is enlarged, the left border of dulness being 12.5 cm. to the left of the median line; the right border, 6 cm. to the right. At the bases of the lungs there is dulness and diminished resonance and breath sounds. There is some fluid in the abdomen.

The urine was acid; specific gravity 1.018 to 1.022; glucose absent; albumin present in large amounts, with many hyaline and granular casts and many red blood cells. Wassermann weakly positive.

December 3, a note says: "Patient lying flat in bed in no distress. Hands are blue; face neither cyanotic nor edematous. Heart sounds of good quality; no murmurs. Second sound is reduplicated; second aortic sound much accentuated. Lungs: Dulness at bases extends on both sides to within three finger-breadths of scapulae. There are a few coarse râles at the left base. Abdomen much distended and dull in right flank. No shifting dulness or fluid wave. There is marked edema of body wall from costal margin downward, and of legs and feet, and slight edema of thighs and genitalia." The phthalein test was 48 per cent. December 3. Blood pressure: 160 mm. systolic; 95 mm. diastolic. Patient was in the calorimeter December 6.

December 6, before he was put in the calorimeter, the temperature began to rise and continued irregular, reaching 103 F.; pulse 74 to 100; respirations 18 to 28. The patient developed a cough and the lungs showed many râles but no consolidation. December 8 the alveolar CO<sub>2</sub> was 31 mm. Hg. December 13 the febrile attack ended and patient improved steadily, but on February 4, when he was discharged, he still complained of swelling of the ankles and slight headache. The table of clinical data (Table 6), December 4 to 6, shows the urine volume 800 to 1,300 c.c., with 8 to 11 gm. nitrogen.

CASE 5.—Jack K., aged 28, baker. Admitted Feb. 15, 1916; discharged improved; chronic interstitial nephritis.

*History.*—At the age of 7 the patient had diphtheria. In 1901, while in the army, he had malaria. He has had frequent sore throats and in 1912 had rheumatic fever following tonsillitis. The same year he had urethritis. He was formerly a heavy drinker.

In August, 1914, he began to have pain in the head and lumbar region, epistaxis, vomiting, dizziness, edema of legs, weakness and frequency of urination. He was in the hospital for a long time, being discharged only two months before entrance into Bellevue. Feb. 12, 1916, he had nosebleed, occipital headache and dizziness. Things got black before his eyes. February 15, the day of admission, he noticed edema of the legs. He has been passing urine three to five times at night and has lost 8 pounds in one month. He has had no night sweats or cough.

*Physical Examination.*—A well developed and well nourished man of rather sullen demeanor. He is not dyspneic or cyanotic and there is only slight edema of the feet. Heart: Left border of dulness in the fifth space is 12.5 cm. from the median line; right border, 4 cm. to the right of median line. Radial arteries are hardened. Lungs are clear. Eyes: Fundi show slight haziness of disk outline and of vessels.

March 4 the patient was in the calorimeter. His condition was practically unchanged except that the edema had disappeared.

His urine was acid; specific gravity 1.010 to 1.012 on repeated examinations and in two-hour day and night specimens. Glucose was absent. Albumin was present in a heavy cloud, with casts, a few leukocytes, but no red blood cells. The Wassermann test on the blood was negative.

The blood pressure was, systolic, between 214 and 197; diastolic, between 96 and 145.

Phthalein Tests: February 19, 13 per cent.; March 3, 8 per cent.

Blood: February 23, nonprotein nitrogen 90.7 mg. March 3, nonprotein nitrogen 72.5 mg.; urea nitrogen, 53.9 mg.; creatinin, 2.6 mg.; chlorids, 0.740 per cent.; McLean index, 82. March 2, CO<sub>2</sub> combining capacity of plasma, 53 Vols. per cent.

CASE 10.—Isidore R., aged 26, pedler, Austrian Hebrew. Admitted Jan. 15, 1916; died Jan. 27, 1916; chronic interstitial nephritis; uremia.

*History.*—At the age of 9 years the patient had pneumonia; at 22 years, urethritis. In April, 1915, following exposure, he noticed swelling of ankles and face. Two months later the abdomen became swollen and he had anorexia and gastric distress. He went to the Michael Reese Hospital in Chicago, where he was told his blood pressure was 160 mm. He was treated with hot air baths, the ascites disappeared, and he was discharged in three months. Three weeks later the symptoms returned and he entered Mount Sinai Hospital, New York, where he was told his blood pressure was 200 to 210 mm. He left early in December and on December 21 entered Bellevue. Here his phthalein excretion, December 29, was 10 per cent. in two hours. He was transferred to the Metropolitan Hospital, but left after a few days. Previous to his second admission to Bellevue, Jan. 15, 1916, he suffered from dyspnea, edema, headache, oliguria, nausea and vomiting. The cause of his frequent changes of hospital was evidently his restless, insubordinate disposition.

*Physical Examination.*—January 20: A well nourished man in great distress; very pale; lying on two pillows, gasping for air, with marked sighing, deep respirations every five or six breaths. His mucous membranes were pale, his tongue coated, his pupils large and reacting sluggishly to light. Heart: Left border of dullness in the fifth space 1 cm. outside the nipple line; right border, 3 cm. from median line. The sounds are very short and snapping, with a true gallop rhythm. The lungs are clear. The abdomen is tense and tympanic, with no dullness and no tenderness. There is no edema. The fundi show an albuminuric retinitis.

The urine, January 17, was acid; specific gravity 1.018; no glucose; albumin present in large amount; no casts seen and no red blood cells. January 19 the CO<sub>2</sub> combining capacity of the plasma was, in terms of alveolar CO<sub>2</sub>, only 17 mm. Hg; January 21, only 19 mm. Since he refused all medication by mouth, he was given intravenously 400 c.c. of a 5 per cent. solution of sodium bicarbonate at 8 p. m., January 21. Half an hour later he had a chill and the pulse became very rapid. Next day the respirations, which had been rapid, shallow and periodic, became normal. The CO<sub>2</sub> capacity rose to 37 mm. Hg. He was brighter mentally, though still apathetic. A marked diarrhea developed. On the 23d, a pericardial rub was heard and the patient became more stuporous. He was in the calorimeter January 24. On the 27th he died. No necropsy could be obtained.

January 15 to 19 the temperature was 99 to 101; pulse 88 to 96; respirations 20 to 24; stools, one a day. January 20 to 27 the temperature was 100 to 101; pulse 92 to 110; respirations 12 to 36; stools, five to ten a day. The blood examinations were as follows:

January 20, nonprotein nitrogen, 103 mg.; chlorids 0.609 per cent. January 22, nonprotein nitrogen, 101 mg.; chlorids, 0.572 per cent. January 24, nonprotein nitrogen, 165 mg.; urea nitrogen, 68 mg.; chlorids, 0.609 per cent.

The CO<sub>2</sub> combining capacity of the blood plasma in volumes per cent. was as follows: January 19, 25; January 20, 29; January 21, 28; January 22, 55; (after bicarbonate) January 23, 53; January 24, 44; January 26, 51; January 27, 44.

The systolic and diastolic blood pressures were:

	Systolic, Mm.	Diastolic, Mm.
January 18.....	210	110
January 21.....	183	110
January 22.....	153	88
January 23.....	158	116
January 24.....	155	100

The patient took very small amounts of food and was incontinent so much of the time that it was impossible to collect twenty-four-hour specimens of urine.



TABLE 4.—NEPHRITIS—

Case Number Name and Date	Temperature		Food			Food N., Gm.	Urine N., Gm.	Excreta N., <sup>1</sup> Gm.
	Max.	Min.	Total Calories	Carbohy- drate, Gm.	Fat, Gm.			
Case 1 Joseph U.								
3/29/16	99.4	97.8	1,081	145	42	3.9	12.1	12.5
3/30/16	99.0	97.4	1,287	176	49	4.4	11.1	11.6
3/31/16	98.8	97.8	1,551	225	54	4.9	13.2	13.7
4/ 1/16	Normal		1,554	215	50	5.0	11.3	11.8
4/ 2/16	Normal		1,553	213	50	5.2	11.4	11.9
4/ 3/16	Normal		1,579	216	61	5.1	9.6	11.1
4/ 4/16	Normal		1,455	207	52	4.8	9.7	10.1
4/ 5/16	Normal		1,200	164	53	5.4	9.1	9.6
4/ 6/16	Normal		1,712	226	64	7.4	9.8	10.5
4/ 7/16	Normal		1,474	207	54	4.9	9.5	10.0
4/ 8/16	98.6	97.0	1,673	228	56	5.0	9.2	9.7
4/ 9/16	Normal		1,433	197	55	5.0	9.3	9.8
4/10/16	Normal		1,375	186	51	5.2	9.3	9.9
4/11/16	105.0	99.4	1,252	182	42	4.4	11.2	11.6
4/12/16	104.8	100.4	0 <sup>2</sup>	0	0	0	7.8 <sup>3</sup>	7.8
4/13/16	102.8	99.6	609	31	45	2.6	4.7 <sup>4</sup>	5.0
4/14/16	103.8	100.6	0 <sup>2</sup>	0	0	0	2.4 <sup>5</sup>	2.4
4/15/16	101.4	98.8	672	61	38	2.8	1.1	2.4
4/16/16	Normal		916	116	40	2.7	2.0	2.3
4/17/16	Normal		830	88	42	3.3	2.6	2.9
4/18/16	Normal		930	88	49	4.3	2.8	3.2
4/19/16	Normal		908	117	38	2.9	3.2	3.5
4/20/16	Normal		692	132	13	1.2	6.5	6.6
4/21/16	Normal		916	149	27	2.1	6.5	6.7
Case 6 Frank O.								
2/27/16	Normal		989	124	43	3.2	15.6	15.9
2/28/16	Normal		664	60	39	2.1	11.9	12.2
3/24/16	Normal		1,687	250	60	4.2	17.5	17.9
3/25/16	Normal		792	108	32	1.8	15.7	15.9

1. Excreta nitrogen calculated as urine nitrogen plus 10 per cent. of food nitrogen. \*

2. Vomited.

3. 26 hours 20 minutes.

4. 21 hours 40 minutes.

5. 24 hours 10 minutes.

## —CLINICAL DATA

Nitrogen Balance, Gm.	Body Weight, Kg.	Urine Volume, C.c.	Calories per Kg.	Fluid Intake, C.c.	NaCl Intake, Gm.	Urine NaCl, Gm.	Blood Pressure, Syst. Diast.	
—8.6	75.7	1,520	14	1,020	1.34	4.08	175	108
—7.1	74.7	1,470	16	1,235	1.10	4.78	165	113
—8.8	73.5	1,682	21	1,470	1.44	4.91	168	110
—6.8	72.9	1,340	21	1,470	1.27	3.84		
—6.7	72.1	1,230	21	1,420	1.39	3.36		
—6.1	71.2	1,090	22	2,050	1.34	3.17		
—5.4	71.4	1,030	20	1,970	1.69	2.19		
—4.3	70.8	946	17	1,380	2.09	1.84	144	84
—3.1	71.4	1,056	24	1,680	2.09	2.03		
—5.0	71.8	995	20	1,400	1.31	1.66		
—4.7	70.8	1,000	23	1,725	1.77	1.66		
—4.8	71.3	1,040	20	2,020	1.74	1.84		
—4.6	70.6	935	19	2,150	1.97	2.30		
—7.2	72.1	1,090	17	2,375	1.33	1.18	150	94
—7.8	....	750 <sup>3</sup>	0	1,360	0	0.52		
—2.4	71.2	480 <sup>4</sup>	8	1,380	0.88	0.52	134	90
—2.4	....	250 <sup>5</sup>	0	2,275	0	0.09		
+0.4	....	160	9	1,000	0.85	0.22		
+0.4	....	290	13	820	0.92	0.40	150	90
+0.4	71.0	350	11	1,005	1.02	0.40		
+1.1	71.0	360	12	1,515	1.54	0.26		
+0.5	70.9	420	12	1,200	1.05	0.31		
—5.4	71.1	400	9	760	0.52	0.45		
—4.6	70.9	380	12	950	0.77	0.36		
—12.7	51.0	2,340	19	2,010	....	4.03	220	145
—10.0	50.2	2,292	13	2,360	0.70	1.86		
—13.7	49.4	4,260	34	1,090	1.18	7.72		
—14.1	48.7	2,930	16	3,270	0.43	4.82		

TABLE 5.—NEPHRITIS.

Case Number, Name and Date	Food			Food N., Gm.	Urine N., Gm.	Excreta N., <sup>1</sup> Gm.	Nitrogen Balance, Gm.
	Total Calories	Carbohy- drate, Gm.	Fat, Gm.				
Case 4 Adam P. <sup>2</sup>	.....	...	..	...	....	....	.....
1/13/16	1,608	183	79	4.6	11.0	11.5	-6.9
1/14/16	1,606	148	95	4.6	7.7	8.2	-3.6
1/15/16	1,627	203	72	5.0	9.3	9.8	-4.8
1/16/16	1,609	203	72	4.4	8.1	8.5	-4.1
1/22/16	1,174	154	47	4.1	7.6	8.0	-4.0
1/23/16	1,464	204	53	5.2	8.2	8.7	-3.5
1/24/16	1,345	175	53	5.1	7.2	7.7	-2.6
1/25/16	1,616	224	61	5.1	7.7	8.2	-3.1
1/26/16	1,595	205	66	5.5	7.9	8.4	-2.9
1/27/16	1,391	186	54	5.0	6.6	7.1	-1.1
1/28/16	1,486	214	51	5.3	6.9	7.4	-2.1
1/29/16	1,434	179	62	4.8	6.0	6.5	-1.7
1/30/16	1,577	226	57	4.6	7.0	7.5	-2.9
1/31/16	1,601	216	63	5.3	7.9	8.4	-3.2
2/ 1/16	1,423	204	52	4.2	6.4	6.8	-2.6
2/ 2/16	1,518	202	60	5.0	6.2	6.7	-2.7
2/ 3/16	1,634	225	63	4.9	6.7 <sup>3</sup>	7.2	-2.3
2/ 4/16	1,488	211	54	4.3	6.5 <sup>4</sup>	7.0	-2.2
2/ 5/16	1,569	225	56	4.9	5.6 <sup>5</sup>	6.1	-1.2
2/ 6/16	1,477	194	60	4.9	6.7 <sup>6</sup>	7.2	-2.3
2/ 7/16	1,321	181	48	5.2	6.7	7.2	-2.0
2/ 8/16	1,558	221	56	4.8	6.6	7.0	-2.2
2/ 9/16	1,529	212	57	4.8	6.5	7.0	-2.1
2/10/16	2,153	195	103	15.2	7.0	8.5	+6.7
2/11/16	2,138	192	105	14.6	10.4	11.9	+2.7
2/12/16	2,024	204	87	14.9	11.1 <sup>7</sup>	12.6	+2.3
2/13/16	2,029	199	86	15.1	12.2 <sup>8</sup>	13.7	+1.4
2/14/16	2,010	191	90	15.2	13.7	15.1	+0.1

1. Excreta nitrogen calculated as urine nitrogen plus 10 per cent. of food nitrogen.

2. Temperature normal.

3. 25 hours 15 minutes.

4. 22 hours 25 minutes.

5. 23 hours 25 minutes.

6. 24 hours 55 minutes.

7. 22 hours 20 minutes.

8. 25 hours 35 minutes.



## CLINICAL DATA

Body Weight, Kg.	Urine Volume, C.c.	Calories per Kg.	Fluid Intake, C.c.	NaCl Intake, Gm.	Urine NaCl, Gm.	Blood Pressure Syst. Diast.	
.....	.....	..	.....	....	....	230	140
61.4	1,590	26	.....	2.57	7.48	226	200
60.6	1,280	26	.....	1.20	4.78		
60.3	1,460	26	1,392	1.68	7.77		
58.3	1,040	27	1,518	0.64	5.39		
54.6	960	22	820	1.18	6.28		
54.6	1,460	27	1,220	1.22	13.34		
54.3	975	25	980	1.39	3.61		
53.7	940	30	810	1.99	3.76		
58.8	1,270	29	1,215	2.00	6.23		
53.7	900	26	820	1.24	3.83		
53.2	1,010	27	1,255	1.68	3.15		
53.6	980	26	1,050	1.71	2.02		
53.9	930	29	1,550	1.34	1.88		
54.8	1,140	29	1,314	2.12	2.30		
54.7	1,410	26	1,540	1.48	1.62	205	130
55.0	1,180	27	1,175	1.54			
55.1	960	29	1,085	2.01	1.91	195	120
54.8	1,340	27	970	1.36	3.17		
53.6	1,280	29	995	1.87	2.88		
53.3	1,360	27	800	1.38	5.65		
52.5	1,337	25	1,060	1.36	4.08	185	120
52.6	1,425	29	1,185	1.66	3.83		
52.4	1,200	29	925	1.40	4.79		
51.6	840	41	1,000	3.39	4.70		
51.7	1,000	41	1,290	3.23	3.64	193	128
51.6	1,000	39	1,040	2.96	5.55		
51.5	1,040	39	1,060	2.90	4.61		
51.7	1,120	38	1,160	2.80	4.98		

TABLE 6.—NEPHRITIS.

Case Number Name and Date	Temperature		Food			Food N., Gm.	Urine N., Gm.	Excreta N., <sup>1</sup> Gm.
	Max.	Min.	Total Calories	Carbohy- drate, Gm.	Fat, Gm.			
Case 2 Lee H. 12/ 4/15	98.0	97.6	1,153	116	56	6.3	7.2	7.8
1/ 5/16	100.6	98.6	1,146	136	51	4.6	7.8	8.3
1/ 6/16	101.8	98.2	447	32	27	3.2	6.8 <sup>2</sup>	7.1
1/ 7/16	102.0	98.6	296	14	20	2.5	16.8 <sup>3</sup>	17.1
1/ 8/16	103.4	99.8	477	45	20	4.0	16.3	16.7
1/ 9/16	103.0	99.6	544	49	27	3.8	14.6	15.0
1/10/16	102.6	100.6	664	68	31	4.0	14.1	14.5
1/11/16	102.4	100.6	142	12	8	0.7	11.3	12.0
1/12/16	102.0	98.6	412	46	18	2.3	11.1	11.3
Case 9 William S. 12/4/15	....	....	1,268	140	55	7.2	9.6	10.3
12/5/15	99.2	97.0	1,180	141	43	8.0	7.6	8.4
12/6/15	103.0	99.8	1,470	144	60	9.4	11.1 <sup>4</sup>	12.0
Case 7 John C. 2/ 8/16	Normal							
2/ 9/16	Normal		1,415	189	57	4.4	8.1	8.5
2/10/16	Normal		2,009	235	76	5.3	6.1	6.6
2/11/16	Normal		1,959	269	78	5.2	5.9	6.4
Case 5 Jack K. 9/4/16	.....		1,569	204	66	4.7	6.8	7.3

1. Excreta nitrogen calculated as urine nitrogen plus 10 per cent. of food nitrogen.

2. 23 hours.

3. 25 hours.

4. 23 hours 25 minutes.

CASE 6.—Frank C., aged 19, born in Italy. Admitted Feb. 23, 1916; died March 27, 1916; chronic interstitial nephritis; uremia.

*History.*—No cause of nephritis was found. He says he has always been well and has been a very moderate user of alcohol. For many years he has had nocturia; for two and a half months dimness of vision; for two days, severe frontal headache and scanty urine; for one day, nausea and vomiting. He has had no edema.

*Physical Examination.*—Patient is well developed and well nourished. He is dyspneic, orthopneic, pale; his eyes have a vacant stare, and he is evidently in pain. Breath urinous. He has no edema. Heart: Left border of dulness in the fifth space 1 cm. outside the nipple line; right border at the sternal margin; no murmurs; second aortic sound markedly accentuated. Arteries not markedly sclerosed. Fundi show marked albuminuric retinitis with hemorrhages. He cannot count fingers at 2 feet.

The urine contained a large amount of albumin, many hyaline and granular casts and a few red blood cells. The Wassermann reaction was very faintly positive.

February 24 he had a spastic convulsion lasting forty-five minutes.

## CLINICAL DATA

Nitrogen Balance, Gm.	Body Weight, Kg.	Urine Volume, C.c.	Calories per Kg.	Fluid Intake, C.c.	NaCl Intake, Gm.	Urine NaCl, Gm.	Blood Pressure, Syst. Diast.
-1.5	55.1	570	21	.....	4.30	....	110 80
-3.7	....	260	..	1,278	2.20	0.85	115 85
-3.4	61.5	240	7	980	0.23	Trace	
-14.6	....	600	..	1,160	0.97	Trace	
-12.7	60.7	940	8	1,140	0.56	Trace	
-12.0	60.2	940+	9	745	0.92	Trace	
-10.5	59.1	900+	11	880	1.56	Trace	
-11.3	59.1	640+	2	885	0.39	Trace	
-9.0	55.9	616	7	720	0.65	Trace	
-3.1	....	1,360	..	.....	4.24		
-0.4	64.1	780	18	.....	7.72	....	155 120
-2.6	64.2	1,278	21	.....	4.40	....	178 90
-4.1	70.0	975	22	955	1.55	5.75	230 125
-1.3	70.1	480	29	850	2.10	1.92	
-0.5	69.3	420	28	975	1.89	2.06	236 130
-8.6	58.5	1,750	26	870	3.23	6.92	197 145

The patient was in the calorimeter February 28. He was irrational and very restless, sitting up in the bed at frequent intervals. His condition had not changed much since admission and he still had headache, but he was passing urine freely, 2,300 to 4,300 c.c. per diem, containing 12 to 17 gm. nitrogen and 2 to 8 gm. sodium chlorid. His breath was not so urinous. The blood pressure was 250 mm. systolic; 130 mm. diastolic.

For a time he improved. March 15 his hemoglobin was 56 per cent.; red blood cells, 3,350,000. March 22 the nonprotein nitrogen of the blood was 46 mg.; creatinin, 1 mg. In the spinal fluid the nonprotein nitrogen was 32 mg.; urea, 17 mg. Blood pressure: 220 mm. systolic; 178 mm. diastolic.

March 21 he began to have headache and nausea once more. March 22 he had a convulsion. After this he became blind, grew more toxic, with marked headaches, and on March 27 died. No necropsy was obtained.

February 28 the urine was collected as follows:

Time of Voiding	Volume, C.c.	Specific Gravity
9:00 a. m.....	410	1.010
2:30 p. m.....	425	1.015
5:20 p. m.....	400	1.010
9:10 p. m.....	300	1.010
4:00 a. m.....	600	1.012
6:30 a. m.....	280	1.010



February 28, McLean index 79. CO<sub>2</sub> combining capacity of the plasma in volume per cent., February 24, 66; February 28, 64.

CASE 7.—John C., aged 61, watchman. Admitted Feb. 8, 1916; transferred to City Hospital Feb. 18, 1916, improved; chronic interstitial nephritis.

*History.*—Thirty-five years prior to test he had urethritis and probably lues. Six years prior he had rheumatic pains in the left shoulder. He has been an excessive pipe smoker but a moderate user of alcohol. In spite of his 61 years he still had the sexual vigor of a young man.

Two years prior to test his joints ached and he was told that his blood pressure was 288 mm. For the five months previous to examination he had been unable to work on account of failing eyesight. He has been dyspneic on climbing two flights of stairs. At times he has severe frontal headaches, dizziness and occasionally loses control of his tongue and becomes aphasic. He has marked frequency of urination, six to eight times in the day, four or more times at night.

*Physical Examination.*—Well nourished; looks younger than his age. His respirations are deep and regular. He is orthopneic but not cyanotic or edematous. The teeth are in very bad condition. Heart: Apex beat in the sixth space 15 cm. from the median line; second aortic sound much accentuated. There is a large, deep scar on the penis.

Urine: February 8 the phthalein output was 25 per cent. in two hours. February 9 to 12 the daily urine volume was 400 to 1,000 c.c.; nitrogen, 6 to 8 gm.; sodium chlorid 2 to 6 gm. His temperature was normal; pulse 60 to 88; respiration 14 to 22. The blood pressure on two examinations was 285 and 236 mm. systolic; 165 and 130 mm. diastolic. February 8 the McLean index was 136 and the CO<sub>2</sub> combining power of the plasma was 69 volumes per cent.

CASE 8.—Mildred C., aged 39, milliner, born in England, single. Admitted Nov. 23, 1915; discharged improved, Jan. 6, 1916. Chronic interstitial nephritis; uremia; hyperthyroidism (?).

*History.*—Three years prior to admission she had diphtheria. In August, 1915, while on a street car, she began to suffer from headache and vomiting and was brought to Bellevue, where she remained unconscious for four days, and was discharged two weeks later. At this time the urine contained much albumin and many hyaline and granular casts. The Wassermann reaction was strongly positive.

As long as she can remember she has had severe headaches, with nausea and vomiting. These come about every month but are not related to the menses, which are normal. Her appetite is fair, bowels constipated. For one year she has had marked nocturia.

Following her first stay in Bellevue she went back to work. For a week she has had a severe headache. She has had no dyspnea and no edema.

*Physical Examination.*—November 23: A thin, nervous woman who looks chronically ill. She is slightly dyspneic and orthopneic. There is moderate exophthalmos but no von Graefe's sign, no goiter, no tremor and only slight tachycardia. Heart: Left border of cardiac dullness is 12.5 cm. from the midsternal line; right border, at right sternal margin; the second aortic sound is accentuated; in the aortic region there is a thrill and a systolic murmur. The lungs are normal. The liver edge, spleen and right kidney are palpable. The knee jerks are exaggerated. Ophthalmoscopic examination, negative.

The urine was acid; specific gravity 1.010 to 1.018; glucose absent; albumin present in large amount; a few granular casts were found but no red blood cells. The blood count showed:

Red blood cells.....	5,080,000
Leukocytes .....	7,200
Differential, normal	

The patient was put on a somewhat restricted diet and given chloral, 5 grains, and potassium iodid, 5 grains, every day except December 10, when she was in the calorimeter. She improved steadily and was able to go back to work January 6.

The temperature was normal; pulse, 85 to 100; respiration, 20 to 22. The systolic and diastolic blood pressures were as follows:

	Systolic, Mm.	Diastolic, Mm.
November 23.....	265	190
December 9.....	260	170
January 5.....	250	150

Phthalein test, December 1, first hour, 10 per cent.

November 30 the blood analysis was as follows:

Sugar .....	105.0	mg.
Chlorids .....	0.54	per cent.
Urea nitrogen .....	25.0	mg.
Ammonia nitrogen .....	3.2	mg.
Uric acid .....	2.6	mg.
Creatinin .....	2.0	mg.
Corpuscular volume .....	36.0	per cent.

December 10 the McLean index was 134.

Feb. 13, 1916, the patient was admitted to Bellevue once more. She had a severe headache the day before admission, followed by a general convulsion. The blood pressure was 240 mm. systolic; 128 mm. diastolic. A few days later she left the hospital in fair condition.

477 First Avenue.

## CLINICAL CALORIMETRY

### TWENTY-THIRD PAPER

#### THE EFFECT OF ROENTGEN-RAY AND RADIUM THERAPY ON THE METABOLISM OF A PATIENT WITH LYMPHATIC LEUKEMIA \*

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In this paper there will be presented the results of calorimetric observations made in a case of chronic lymphatic leukemia, both before and after vigorous Roentgen-ray therapy, and also after exposure to radium. The basal metabolism in leukemia will be discussed and the effect of irradiation thereon.

The literature contains but few observations of this sort. Those by Grafe,<sup>1</sup> Magnus-Levy,<sup>2</sup> Kraus,<sup>3</sup> and Bohland<sup>4</sup> being the only ones that we have found suitable for comparison with our own case.

By referring to Table 1, in which the experiments of these several authors are collected,<sup>5</sup> it will be seen that in both forms of leukemia there is invariably a marked rise in the basal metabolism expressed in terms of body surface area. This is found in both types about equally, the average increase over the normal in eight cases of lymphatic leukemia being 52 per cent., and in five of the myelogenous type, 44 per cent.<sup>6</sup>

The cause of the increase is uncertain; Grafe believed it to be due to the active metabolism of the large numbers of premature white

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1. Grafe, E.: Die Steigerung des Stoffwechsels bei chronischer Leukämie und ihre Ursachen, *Deutsch. Arch. f. klin. Med.*, 1911, **102**, 406.

2. Magnus-Levy, A.: Ueber den Stoffwechsel bei acuter und chronischer Leukämie, *Virchows Arch. f. path. Anat.*, 1898, **152**, 107.

3. Kraus, F.: Ueber den Einfluss von Krankheiten, besonders von anämischen Zuständen, auf den respiratorischen Gaswechsel, *Ztschr. f. klin. Med.*, 1893, **22**, 449.

4. Bohland, K.: Ueber den respiratorischen Gaswechsel bei verschiedenen Formen der Anämie, *Berl. klin. Wehnschr.*, 1893, **30**, 417.

5. A more extended form of this table will be found in Paper 15 of this series, *THE ARCHIVES INT. MED.*, 1916, **17**, 974.

6. In this discussion the patients S. W. of Kraus and H. of Bohland were omitted, there being some uncertainty as to which type of leukemia they belonged.



blood cells. He based this belief on observations made on the metabolism of leukemic blood which he carried out by several methods, chiefly by Bohr's blood gas pump or the Haldane-Barcroft blood gas analysis apparatus. Calculating from such experiments, he found that in individual cases the oxygen demand of the total number of leukocytes in the body might form as much as 10 per cent. of the patient's total oxygen requirement.

The intensity of the rise in basal metabolism, Grafe found, ran parallel with the clinical severity of the disease.

The total nitrogen metabolism has been studied by numerous investigators. Such observations as those of von Noorden,<sup>7</sup> Magnus-Levy,<sup>2</sup> Taylor,<sup>8</sup> Musser and Edsall,<sup>9</sup> Edsall,<sup>10</sup> Goodall,<sup>11</sup> Stejskal and Erben,<sup>12</sup> Döri<sup>13</sup> and of Cavina<sup>14</sup> seem to show fairly definitely that there is, in chronic leukemia, no increase in nitrogenous metabolism at all comparable with that in the respiratory metabolism. There is either a slight retention, a slight loss, or a balance. In acute leukemia, on the other hand, as shown by Magnus-Levy's and Edsall's cases, there is an enormous increase in protein metabolism. Edsall's patient had a negative nitrogen balance of 22.28 gm. a day while on a nitrogen intake of 7.25 gm.!

A marked increase in the uric acid output has been found by a number of observers. One of Magnus-Levy's cases, on a low food intake, excreted 12.2 gm. of uric acid. Wende<sup>15</sup> reports a case of lymphatic leukemia with a uric acid excretion of 5 gm. The case of acute leukemia of Edsall and the two cases of chronic leukemia of Musser and Edsall all showed an increased uric acid output. Lossen and Morawitz<sup>16</sup> also report two cases with high uric acid elimination.

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7. Von Noorden, C.: *Lehrbuch der Pathologie des Stoffwechsels*, Berlin, 1893, p. 349.

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9. Musser, J. H., and Edsall, D. L.: *Study of the Metabolism in Leukemia, Under the Influence of the X-Ray*, Tr. Assn. Am. Phys., 1905, **20**, 294.

10. Edsall, D. L.: *Case of Acute Leukemia, with Some Striking Clinical Features*, Tr. Assn. Am. Phys., 1905, **20**, 279.

11. Goodall, H. W.: *Nitrogenous Metabolism in a Case of Chronic Myelogenous Leukemia*, Boston Med. and Surg. Jour., 1914, **170**, 789.

12. Stejskal, C. v., and Erben, F.: *Stoffwechselversuch bei lymphatischer und lienalmyelogener Leukämie*, Ztschr. f. klin. Med., 1900, **39**, 151.

13. Döri, B.: *Stoffwechseluntersuchungen bei einer mit Benzol behandelten chronischen leukämischen Myelose*, Wien klin. Wchnschr., 1913, **26**, 2034.

14. Cavina, G.: *Untersuchungen über den Stoffwechsel bei der lymphatischen Leukämie während der Röntgenbestrahlung*, Deutsch. Arch. f. klin. Med., 1913, **110**, 585.

15. Wende, G. W.: *Case of Lymphatic Leukemia*, Am. Jour. Med. Sc., 1901, **122**, 836.

16. Lossen, J., and Morawitz, P.: *Chemische und histologische Untersuchungen an bestrahlten Leukämikern*, Deutsch. Arch. f. klin. Med., 1905, **83**, 288.

TABLE 1.—BASAL METABOLISM IN CASES OF

Observer	Type	Patient	Calories per Square Meter per Hour (Meeh)
Grafe, E. ....	Lymphatic.....	{ T. S. 1a .....	68.7
		{ T. S. 1b .....	52.0
		{ T. S. 1c .....	48.7
	Lymphatic.....	G. K. 2.....	36.8
	Lymphatic.....	{ C. L. 3a .....	44.5
		{ C. L. 3b .....	46.9
	Lymphatic.....	M. T. 4.....	65.0
	Lymphatic.....	M. D. 5.....	49.8
	Myelogenous.....	F. H. 6.....	53.2
	Myelogenous.....	M. H. 7.....	49.8
Magnus-Levy.....	Myelogenous.....	H. B. 8.....	42.3
	Lymphatic.....	J. ....	45.1
	Spleno-myelogenous.....	M. B. ....	48.7
		M. B. ....	46.4
M. B. ....		45.8	
Kraus, F. ....	Spleno-myelogenous.....	{ W. K. ....	47.8
		{ W. K. ....	46.7
		{ W. K. ....	49.4
	Lienalis.....	{ S. W. ....	49.7
		{ S. W. ....	49.2
	Lienalis.....	{ H. ....	51.8
		{ H. ....	57.8
		{ H. ....	56.7
		{ H. ....	54.8
	Bohland, K. ....	Lymphatic.....	{ R. ....
{ R. ....			42.1
{ R. ....			42.9
Lymphatic and Lienalis.....		{ L. ....	48.0
		{ L. ....	43.7
		{ L. ....	42.9
		{ L. ....	59.0
		{ L. ....	47.3
		{ L. ....	47.3

\* For normal averages those given in Paper 13 of this series (Gephart and Du Bois, THE ARCHIVES INT. MED., 1916, 17, 918) were used

## LEUKEMIA COLLECTED FROM THE LITERATURE

Per Cent. Above or Below Average Normal*	Total Leukocytes	Per Cent. Polymorpho- nuclears	Per Cent. Leukocytes	Total Red Cells	Per Cent. Hemoglobin
+123	820,000	10	89	3,600,000	40
+69	205,000	10	89		
+58	200,000	5	95	2,400,000	30
+19	40,000	28	72	4,800,000	90
+55	130,000	4	96		
+63	165,000	2	98	3,640,000	75
+87	32,000	12	87	3,800,000	70 to 75
+62	170,000	2	98	3,800,000	80
+53	140,000	95	5	4,700,000	60
+54	180,000	95	5	2,600,000	55
+22	230,000	98	2	.....	75
+30					
+58	450,000	..	..	2,800,000	50
+51					
+43					
+88	230,000	..	..	2,400,000	45
+85					
+42					
+54	345,000	..	..	2,990,000	50
+52	to 425,000	..	..	to 2,720,000	to 45
+60	Ratio of Whites to Reds = 1:5				
+79					
+76					
+70					
+59	Ratio of Whites to Reds = 1:50				
+21					
+24					
+38	Ratio of Whites to Reds = 1:10				
+26					
+24					
+70					
+36					



In Goodall's case there was a high endogenous uric acid elimination, but this showed marked variation which had no relation to the total nitrogen or to the apparent leukocyte destruction. Goodall concluded that the destructive process might be carried beyond the uric acid stage, and that, also, retention of uric acid might occur for the purpose of new leukocyte formation. Other writers, among them Stejskal and Erben,<sup>12</sup> and Rzentkowski,<sup>17</sup> have found no increase in the uric acid output in chronic leukemia. The latter not only failed to find any increase in the endogenous uric acid elimination, but also on feeding meat, Liebig's extract, or uric acid, failed to get an increase in the exogenous elimination. This suggested that the process of uricolysis was increased over the normal.

Folin and Denis<sup>18</sup> have found that the blood uric acid is markedly increased in leukemia.

A retention of phosphates in leukemia has been found by several writers, Moraczewski,<sup>19</sup> Stejskal and Erben,<sup>12</sup> and Musser and Edsall.<sup>9</sup> The latter believe this retention to be due to the rapid building of leukemic tissue, a tissue especially rich in phosphorus.

Stejskal and Erben found a loss of calcium in their case of lymphatic leukemia.

There seems to be, therefore, plenty of evidence to show that a profound change in the general metabolism may occur in leukemia. Since this is so, and since Roentgen and radium therapy have been used very extensively of late in both types of chronic leukemia, it seemed important to determine the effect of such forms of treatment on the leukemic's basal metabolism.

That the Roentgen ray is capable of producing a change in the metabolism in normal individuals is shown by the experiments of Linser and Sick<sup>20</sup> who found an increase in uric acid and purin bases and a decrease in the leukocytes of the blood after irradiation. Baermann and Linser<sup>21</sup> also found a rise in the total nitrogen. Benjamin and von Reuss,<sup>22</sup> in a normal dog after a single very intense

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19. Moraczewski, W. v.: Stoffwechselversuche bei Leukämie und Pseudo-leukämie, *Virchows Arch. f. path. Anat.*, 1898, **151**, 22.

20. Linser, P., and Sick, K.: Ueber das Verhalten der Harnsäure und Purin-basen im Urin und Blut bei Roentgenbestrahlungen, *Deutsch. Arch. f. klin. Med.*, 1906-1907, **89**, 413.

21. Baermann, G., and Linser, P.: Ueber die lokale und allgemeine Wirkung der Röntgenstrahlen, *München. med. Wchnschr.*, 1904, **51**, 996.

22. Benjamin, E., and v. Reuss, A.: Roentgenstrahlen u. Stoffwechsel, *München. med. Wchnschr.*, 1906, **53**, 1862.

exposure, found a slight rise in the total nitrogen, on a fixed intake, lasting several days, and after the second day an increase in phosphorous pentoxid.

A number of writers, among others Ellis,<sup>23</sup> Morris,<sup>24</sup> Heineke,<sup>25</sup> Helber and Linser,<sup>26</sup> Belot<sup>27</sup> and Harvey<sup>28</sup> have described the histologic action of the Roentgen ray on normal tissues. In general, the effect seems to be a destructive action on the entire hematopoietic system. Such organs as the spleen show a diminution in size and degeneration of the parenchyma after irradiation. The action seems to be particularly active in the case of white blood cells, especially those of the lymphocyte series. The white cells, both in the circulation and in the blood forming organs, are attacked. The nucleus is fragmented first, and after this there is a lysis of the entire cell.

We have not found in the literature any observations on the effect of irradiation on the basal metabolism in normal individuals. The effect of the Roentgen ray has been studied very much more extensively in the case of the leukemic than in that of the normal individual. Since Senn,<sup>29</sup> in 1903, first used Roentgen therapy in leukemia, scores of papers on this subject have appeared. A very extensive bibliography up to 1911 will be found in the paper of Keymling.<sup>30</sup>

The effect of irradiation on the basal metabolism was studied in Grafe's case, T. S. (Table 1). The heat production per square meter per hour having been 68 calories before treatment, fell to 52 calories and later to 48 while under treatment. During this time the leukocyte count fell from 820,000 to 200,000.

The changes in the total nitrogen, uric acid and purin base elimination after Roentgen-ray treatment have been observed by numerous writers. Lossen and Morawitz,<sup>16</sup> in one case of myelogenous leukemia, found that the uric acid elimination, having been high during irradiation

23. Ellis, A. G.: The Pathology of the Tissue Changes Induced by the X-Ray, *Am. Jour. Med. Sc.*, 1903, **125**, 85-96.

24. Morris, R. S.: Action of Roentgen Rays on the Blood, *Am. Med.*, 1905, **10**, 946.

25. Heineke, H.: Experimentelle Untersuchungen über die Einwirkung der Röntgenstrahlen auf innere Organe, *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1905, **14**, 21.

26. Helber, E., and Linser, P.: Experimentelle Untersuchungen über die Einwirkung der Roentgenstrahlen auf das Blut, *München. med. Wchnschr.*, 1905, **52**, 689.

27. Belot, J.: On the Influence of the X-Ray on the Hematopoietic Organs, *Arch. Röntgen Ray*, 1906, **11**, 67 and 108.

28. Harvey, W. G.: On the Pathological Effects of Röntgen Rays on Animal Tissues, *Jour. Path. and Bacteriol.*, 1908, **12**, 549.

29. Senn, N.: Case of Splenomedullary Leukemia Successfully Treated by the Use of the Roentgen Ray, *Med. Rec.*, New York, 1903, **64**, 281.

30. Keymling, E.: Die Röntgen-therapie der Leukämie, *Ztschr. f. Röntgen u. Radiumforsch.*, 1911, **13**, 306, 337, 428, 453.



tion, returned to normal coincidently with the leukocyte count. In another case of the same disease they found that the uric acid remained high even after irradiation had produced an actual leukopenia. Heile<sup>31</sup> found an increase in both uric acid and purin bases in three cases. Königer,<sup>32</sup> in seven cases of chronic myelogenous leukemia, found a marked change in the uric acid elimination following irradiation. This consisted, first, in a marked increase, and later, in a reduction to a normal figure as the blood picture approached an aleukemic condition. Cavina,<sup>14</sup> on the other hand, found no increase either in uric acid or total nitrogen in a case of lymphatic leukemia while the patient was being treated with the Roentgen ray. The nitrogen balance was invariably positive, although the leukocyte count fell from 60,860 to 24,800.

The cases of Musser and Edsall<sup>9</sup> are especially interesting in this connection. In one of their cases in which the Roentgen ray had no beneficial effect clinically, it likewise had little or no effect on the nitrogenous metabolism, whereas in the other case, in which there was a marked reduction in the number of white cells and clinical improvement, there was a definite increase in uric acid and purin base output, a very striking loss of nitrogen and an increased elimination of phosphorus pentoxid.

The histologic action of the Roentgen ray seems to be essentially the same in leukemia as in normal individuals. It has been described in detail in the papers of Warthin<sup>33</sup> and of Aubertin and Beaujard.<sup>34</sup> The immediate action is that most cells of the hematopoietic organs are either destroyed at once or so damaged that they become so later. This action is especially violent on cells of the lymphatic series. In either the lymphatic or the bone marrow series, however, both young and old cells are attacked, with the result that, although the total number of white cells may be reduced to normal and the blood rendered aleukemic, nevertheless the differential count never becomes normal. Pathologic cells persist even when an absolute leukopenia has been produced. As a number of writers point out, therefore, the treatment is purely palliative, but, owing to the great reduction in the size of the spleen and the general clinical improvement, in many cases is, nevertheless, distinctly worth while.

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31. Heile: Ueber intravitale Beeinflussung autolytischer Vorgänge im Körper, *Ztschr. f. klin. Med.*, 1904, **55**, 508.

32. Königer, H.: Der Einfluss der Roentgenbehandlung auf den Stoffwechsel bei chronischer myeloider Leukämie, *Deutsch. Arch. f. klin. Med.*, 1906, **87**, 31.

33. Warthin, A. S.: The Minute Changes Produced in Leukemic Tissues by Exposure to Roentgen Rays, *Am. Jour. Med. Sc.*, 1914, **147**, 72.

34. Aubertin, C., and Beaujard, E.: Sur le mode d'action de la radiothérapie dans la leucémie myéloïde, *Bull. et mém. Soc. méd. d. hôp. de Paris*, Series 3, 1913, **36**, 66.



The effect of radium on the metabolism has been studied by Kikkaji<sup>35</sup> who found a significant increase in the basal metabolism in a case of chronic arthritis and in a normal man, but found no effect in a second case of chronic arthritis. The two cases that showed a rise in basal metabolism likewise showed an increase in total nitrogen and uric acid elimination.

Histologically, as shown by the researches of Proescher and Almquest,<sup>36</sup> radium has much the same action on the blood-cells and hematopoietic organs as the Roentgen ray.

Let us now sum up the various facts gathered from the literature. In the first place, there is good evidence that the basal metabolism is always increased in leukemia.

The metabolism of leukemic blood is more active than that of normal blood.

The nitrogenous balance is usually negative in acute and variable in chronic leukemia.

The endogenous uric acid elimination and the uric acid content of the blood are generally increased.

There is often a retention of phosphates.

The Roentgen ray may or may not have a profound effect on the metabolism in leukemia. When it does, this effect consists in an increased elimination of nitrogen; also of uric acid, the latter, however, returning to a normal figure as the leukocyte count approaches normal.

When the leukocyte count falls as a result of irradiation, there is some evidence to show that the basal metabolism falls coincidently.

#### METHODS USED

During the period that he was under observation the subject of this research, Anthony W., spent part of the time in the metabolism ward of the Sage Institute, a part at the hospital of the Rockefeller Institute, and a part at Saint Luke's Hospital, New York.

While at the Sage Institute the methods used, both those of calorimetry and the general routine, were as already described in the third and fourth papers of this series.<sup>37</sup> The uric acid in blood and urine

35. Kikkaji, T.: Ueber den Einfluss von Radiumemanation auf den Gesamtstoffwechsel in Organismus, *Radium in Biologie und Heilkunde*, 1911, **1**, 46.

36. Proescher, F., and Almquest, B. R.: Contribution on the Biological and Pathological Action of Soluble Radium Salts, with Special Reference to Its Therapeutic Value in Pernicious Anemia and Leukemia, *Radium*, 1914, **3**, 65.

37. Gephart, F. C., and Du Bois, E. F.: The Organization of a Small Metabolism Ward, *THE ARCHIVES INT. MED.*, 1915, **15**, 829; The Determination of the Basal Metabolism of Normal Men and the Effect of Food, *ibid.*, p. 835.

was determined by Benedict's<sup>38</sup> method. For the calorimeter experiments the steamer chair was used.

The Roentgen-ray treatments were given at the hospital of the Rockefeller Institute. The treatment used varied from that generally employed in that the whole surface of the body was exposed to a radiation of low penetration.

The radium treatments were given by Dr. Karl M. Vogel at St. Luke's Hospital. Radium was applied over the splenic region on six occasions in the months of July, August and September, 1916.

#### HISTORY OF CASE

*History.*—Anthony W., man, aged 59, single, born in Ireland, was admitted to the Sage Metabolism Ward in Bellevue Hospital April 18, 1916 (chronic lymphatic leukemia). He had been in America since 1898.

*Family History.*—Gout on both sides of the family; otherwise negative.

*Past History.*—At the age of 7 he had diphtheria and scarlet fever, which were followed by "dropsy." At 10 years he had typhoid fever. Between the ages of 18 and 25 he had rather severe attacks of migraine. When 33 years old he had pains in the left great toe which, he was told, were due to gout. Four years later he had an attack of sciatica which lasted for six months.

*Present History.*—Symptoms referable to the present trouble had existed for thirteen months before his admission to Bellevue Hospital (March, 1915), when he began to have intermittent attacks of severe pain in the left lower part of the abdomen. The pain was definitely localized above the crest of the ilium and in the groin. It radiated occasionally to the back and left arm. In October, 1915, he began to notice shortness of breath on slight exertion, with a slight orthopnea at night. In December, 1915, he first noticed a firm mass in the left upper quadrant. About the same time he began to have severe cramp-like contractions in the leg muscles as far as the thigh and in the muscles of the hands. They came frequently during the day, lasted two to three minutes, and were relieved by exercise. In the previous thirteen months he had lost about 50 pounds. His appetite and digestion had been good. He had been constipated and had had a moderate nocturia.

*Physical Examination.*—A well developed, but poorly nourished man, lying comfortably in bed. His skin, sclerae and mucous membranes are pale. The lower teeth show moderate pyorrhea. The thyroid is not enlarged. The heart is enlarged; the borders percuss 15 cm. to the left and 4 cm. to the right of the midsternal line. At the apex and along the sternal margin there is a rough systolic murmur, loudest and roughest in the third left interspace. The lungs are normal.

The spleen can be felt as a definitely outlined, firm mass, dull to percussion, filling the entire left, upper quadrant, and causing a marked bulging in the left flank. Its greatest length is 33.5 cm.; greatest width 18.5 cm. It extends from 2 cm. to the right of the midsternal line deep into the flank, from 11 cm. above the costal margin to 4 cm. below the umbilical line. In the median line, near the costal margin, are left two definite notches. The liver dulness extends from the fifth rib to the costal margin. There are a few pea-sized, soft glands posterior to the left sternomastoid muscle. There

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38. Benedict, S. R., and Hitchcock, E. H.: On the Calorimetric Estimation of Uric Acid in Urine, *Jour. Biol. Chem.*, 1915, **20**, 619. Benedict, S. R.: On the Calorimetric Determination of Uric Acid in Blood, *ibid.*, 1915, **20**, 629.

are larger masses in both axillae and a mass the size of an almond in the left groin. Blood pressure, 142 mm. systolic; 105 mm. diastolic.

His appearance at this time is shown by the accompanying photograph (Fig. 1).

## BLOOD ANALYSIS

		Mg. Per 100 C.c.
April 19, 1916.	Urea N. ....	12.3
	Creatinin .....	1.3
	Uric acid .....	6.15
	Glucose .....	102.0
April 24, 1916.	Nonprotein N. ....	68.2
	Uric acid .....	3.0
May 20, 1916.	Uric acid .....	7.18

## URINALYSIS

April 18, 1916.	Light yellow, clear, slightly acid to litmus
	Sp. gr. .... 1.018
	Albumin.....Slight trace
	Indican.....Trace
	Glucose.....Absent
	Few hyaline casts; few leukocytes and red blood cells

*Subsequent Course.*—April 18. Through the kindness of Dr. Alexander Lambert, to whom we wish to express our thanks, the patient was transferred to the metabolism ward of the Sage Institute. Previously he had been on the general medical service of Bellevue Hospital, Second Division.

April 19. First calorimeter observation.

April 24. Second calorimeter observation.

April 25. Transferred to the hospital of the Rockefeller Institute.

April 26. Roentgen therapy begun.

April 29. Following Roentgen-ray treatments on two successive days (25th and 26th), the patient did not feel well yesterday; headache, weakness, bowels constipated. Condition better today, however.

April 30. Transferred to metabolism ward of Bellevue Hospital.

May 1. Third calorimeter observation. Transferred to hospital of Rockefeller Institute.

May 10. With continued Roentgen-ray dosage the total white count has come down almost progressively. A few days previous to entrance it was 55,000. Two days ago it was 22,000. Patient has been symptom-free save for tenderness over the spleen at times.

May 18. Transferred to the metabolism ward in Bellevue Hospital.

May 19. Fourth calorimeter experiment. Transferred to hospital of the Rockefeller Institute.

May 20. Five days ago patient had severe nosebleed. Size of spleen remains stationary. At times patient has considerable pain in left flank with tenderness over spleen.

Since coming to hospital patient has noticed a small, non-tender mass in right groin, more prominent on straining. Examination shows a loose, right inguinal ring with impulse and visible bulge on cough.

Repeated Roentgen-ray exposure has caused tanning of the whole skin, with some erythema and desquamation over back and thighs.

June 10. Another slight nose-bleed yesterday. Patient is exercising a little each day; feels very weak, however.

Percussion dullness of heart less in both directions by 1 cm., and the murmur is loudest in fifth space, midclavicular line.

June 26. The spleen is growing in size and is much firmer, with a more prominent edge than on entrance. It has extended to the right 2 cm. The



liver edge is palpable 1.5 cm. below the costal margin. There are palpable almond-sized lymph nodes in the neck on either side, in axillae and groins.

There is erythema and desquamation of the skin over the left chest in the axillary line due to the Roentgen ray. There are several very tender areas over the spleen.

July 18. Admitted to St. Luke's Hospital for radium treatment. In applying radium the splenic region was divided into four equal parts and each part was subjected to a two-hour application of radium, making each period eight hours in all.

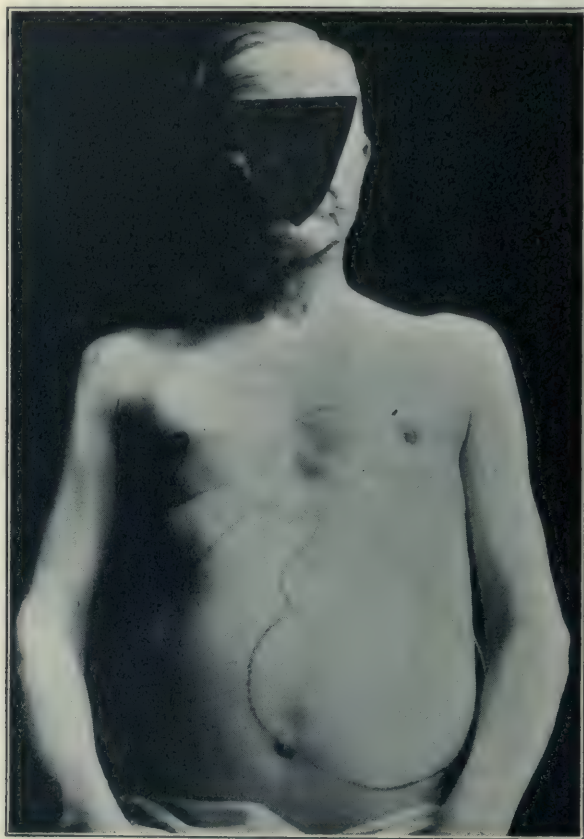


Fig. 1.—Anthony W., April, 1916, before treatment.

July 25. Radium applied.

August 10. Radium applied.

August 17. Radium applied.

August 26. Radium applied.

August 30. Discharged from hospital.

September 9. Radium applied.

September 23. Radium applied.

Oct. 24, 1916, entered metabolism ward at Bellevue Hospital. Since his first radium treatment the patient has had no pain and very little tenderness over the spleen. The spleen has grown smaller and he has felt much better.

He has had less dyspnea and can walk as far as one mile (slowly) without fatigue.

*Physical Examination* shows the heart enlarged to the left, with a soft, blowing systolic murmur at the apex, transmitted to the axilla and toward the sternum. The spleen is distinctly smaller than when he was first observed. His appearance at this time is shown in Figure 2.

October 25. Fifth calorimeter experiment.

Blood Analysis: Uric acid, 3.6 mg. per 100 c.c. of blood.



Fig. 2.—Anthony W., Oct. 25, 1916, after Roentgen and radium treatment.

#### DISCUSSION OF RESULTS

The basal metabolism in a case of chronic lymphatic leukemia showed a rise of 44 per cent. above the average figure for normal men of the patient's age. This finding is similar to those of Grafe, Magnus-Levy, Kraus and Bohland. The high metabolism in leukemia is especially interesting, because (in one case, at least) there was nothing about the general appearance to lead one to suppose that the metabolism was increased. In Graves' disease, as already pointed out

TABLE 2.—LEUKEMIA. DATA OF—

Subject, Date, Weight, Surface Area, Linear Formula	Period	End of Period	Carbon Dioxid, Gm.	Oxygen, Gm.	R. Q.	Water, Gm.	Urine N per Hour, Gm.	Indirect Calo- rimetry, Cal.	Heat Elimi- nated, Cal.
Anthony W. 4/19/16 75.9 Kg. 1.96 Sq. M.	Prelim.	12:00	.....	.....	.....	.....	.....	.....	.....
	1	1:00	36.0	34.3	0.76	57.6	0.311	113.6	115.4
	2	2:00	34.5	33.5	0.75	58.8	0.311	110.5	112.2
	Aver.	.....	.....	.....	.....	.....	.....	.....	.....
Anthony W. 4/24/16 75.4 Kg. 1.96 Sq. M.	Prelim.	12:02	.....	.....	.....	.....	.....	.....	.....
	1	1:02	33.4	34.3	0.71	48.0	0.402	.....	102.8
	2	2:02	33.4	32.6	0.75	55.3	0.402	.....	113.0
	Aver.	.....	.....	.....	.....	.....	.....	100.5	.....
Anthony W. 5/ 1/16 78.8 Kg. 1.97 Sq. M.	Prelim.	11:41	.....	.....	.....	.....	.....	.....	.....
	1	12:31	27.6	26.8	0.75	39.1	0.296	88.4	88.3
	2	1:41	40.1	36.2	0.80	61.5	0.415	121.1	128.5
	Aver.	.....	.....	.....	.....	.....	.....	.....	.....
Anthony W. 5/19/16 75.1 Kg. 1.95 Sq. M.	Prelim.	10:39	.....	.....	.....	.....	.....	.....	.....
	1	11:39	33.2	30.7	0.79	50.0	0.442	101.9	104.1
	2	12:39	34.4	32.0	0.78	56.0	0.442	106.1	110.7
	Aver.	.....	.....	.....	.....	.....	.....	.....	.....
Anthony W. 10/25/16 77.8 Kg. 1.98 Sq. M.	Prelim.	11:55	.....	.....	.....	.....	.....	.....	.....
	1	12:56	30.4	26.1	0.85	46.0	0.592	.....	94.5
	2	1:55	31.3	31.0	0.74	59.2	0.592	.....	103.1
	Aver.	.....	.....	.....	.....	.....	.....	94.4	.....

TABLE 3.—LEUKEMIA.—

Name and Date	Food				Food N., Gm.	Urine N., Gm.
	Total Calories	Calories per Kg.	Carbohy- drate, Gm.	Fat, Gm.		
Anthony W. 4/19/16	1,899	25	144	110	11.0	10.2
4/20/16	2,534	33	212	138	14.9	10.3
4/21/16	2,459	32	215	132	13.7	11.8
4/22/16	2,611	34	214	144	15.2	10.4
4/23/16	2,524	33	196	142	15.5	10.9
4/24/16	2,276	..	175	128	14.2	12.0
5/ 1/16	2,037	..	160	113	12.9	10.2

\* Excreta nitrogen calculated as urine nitrogen plus 10 per cent. of food nitrogen.



## —CALORIMETER OBSERVATIONS

Direct Calo- rimetry (Rectal Temp.), Cal.	Rectal Temp., C.	Aver- age Pulse	Work- Adder, Cm.	Non- protein R. Q.	Per Cent. Calories from			Calories per Hour		Remarks
					Pro- tein	Fat	Carbo- hyd.	Per Kg.	Per Sq. M.	
.....	37.5	..	..	....	..	..	..	....	....	Basal
110.5	37.4	73	60	0.76	..	..	..	....	....	Restless
108.2	37.4	71	36	0.74	..	..	..	....	....	Quiet
.....	.....	..	..	0.75	7	79	14	1.48	57.1	
.....	37.5	..	..	....	..	..	..	....	....	Basal
104.2	37.5	60	32	0.70	..	..	..	....	....	Quiet; work-adder very sensitive
110.6	37.5	62	33	0.74	..	..	..	....	....	Quiet; work-adder very sensitive
.....	.....	..	..	0.72	10	86	4	1.45	55.9	
.....	37.3	..	..	....	..	..	..	....	....	Basal
79.6	37.2	62	31	0.74	..	..	..	....	....	50 min. period; very quiet
135.1	37.3	59	44	0.80	..	..	..	....	....	70 min. period; fairly quiet
.....	.....	..	..	....	9	68	23	1.36	53.2	Per period
.....	37.2	66	..	....	..	..	..	....	....	Basal
98.5	37.1	65	36	0.79	..	..	..	....	....	Slightly restless
113.3	37.2	65	31	0.78	..	..	..	....	....	Slightly restless
.....	.....	..	..	....	11	66	23	1.36	53.4	
.....	37.1	..	..	....	..	..	..	....	....	Basal
93.3	37.1	53	32	....	..	..	..	....	....	Fairly quiet (61 min. period)
100.7	37.1	55	38	....	..	..	..	....	....	Restless; coughed (50 min. period)
.....	.....	..	..	0.78	17	62	21	1.21	47.7	

## —CLINICAL DATA

Excreta* N., Gm.	Nitrogen Bal- ance, Gm.	Uric Acid, Gm.	Purin N., Gm.	Body Weight, Kg.	Urine Volume, C.c.	Blood Pressure, Syst.-Diast.
11.3	-0.3	.....	.....	75.9	1,140	168-95
11.8	+3.1	0.522	0.014	76.2	1,220	
13.2	+0.5	0.520	0.017	76.1	1,300	
11.9	+3.3	0.489	0.006	75.1	1,300	
12.5	+3.0	0.500	0.005	76.5	1,310	
13.4	+0.8	0.563	0.038	75.4	1,350	140-98
11.5	+1.4	0.569	0.016	76.8	1,666	

in the Fourteenth Paper of this series, there are a number of manifestations—the profuse sweating, the hot, flushed skin, the tachycardia, etc.—which are probably due to the high metabolism. Practically all such signs were lacking in this leukemic. His skin was not hot or flushed; he had no tachycardia; he may possibly have had a tendency to sweat rather more easily than a normal individual. Moreover, there was no nervous element. He was perfectly placid, was rather interested in the experiments, was not in the least worried by being placed in the calorimeter, and was fairly quiet in all the observations. Nevertheless, he had a very high metabolism. The results of the calorimeter experiments are collected in Table 2.

This increased metabolism was apparently not due to increased combustion of body protein, for the patient was in nitrogen equilibrium on a nitrogenous intake of 13 to 15 gm. per day, and, moreover, he was maintaining his weight on an intake of 2,400 calories. The figures for food and urine analysis are given in Table 3.

An inspection of the respiratory quotients shows them to be rather lower than those of normal individuals who have fasted sixteen hours, but no lower than those of many patients with Graves' disease<sup>39</sup> with a similar increase in metabolism. These low quotients are probably due, as in Graves' disease, to the more rapid consumption of the glycogen store because of the high metabolism.

As to what causes the increased metabolism in leukemia we can not venture an opinion. Grafe thought it was the result of the very active metabolism of young white cells. His experiments on the metabolism of blood do show an increased oxygen consumption in leukemic blood, but he could account for only about 10 per cent. of the total oxygen consumption as being due to leukocytes. Just how he could explain a rise of 40 or 60 per cent. in the basal metabolism on that ground, we do not quite understand.

Grafe's supposition that the high metabolism resulted from the leukocytes, led us to plot the heat production and leukocyte counts of our patient and then, from the literature given in Table 1, to see whether there was any relation between the leukocyte count and the level of the metabolism. Among different cases there was no such relation, but in individual cases there was evidence of a certain parallelism. As the leukocyte count fell, the metabolism did likewise, and vice versa. But the fall in leukocytes is always very much greater than that in the metabolism. This statement applies to Grafe's cases T. S. and C. L., and also possibly to a slight extent to our case, as may be seen by consulting Figure 3. Of course, the lack of relationship between metabolism increase and leukocyte count in different cases

39. Du Bois, E. F.: Metabolism in Exophthalmic Goiter, *THE ARCHIVES INT. MED.*, 1916, **17**, 915.

does not in the least disprove Grafe's theory, for it is rather the rate of production of white cells and not their total number that is the determining factor. Further work on the metabolism of leukocytes could be done to advantage.

Let us now consider what effect the Roentgen rays had on the metabolism. Unfortunately, in this connection we have observations on the basal metabolism only. The data on the nitrogen metabolism

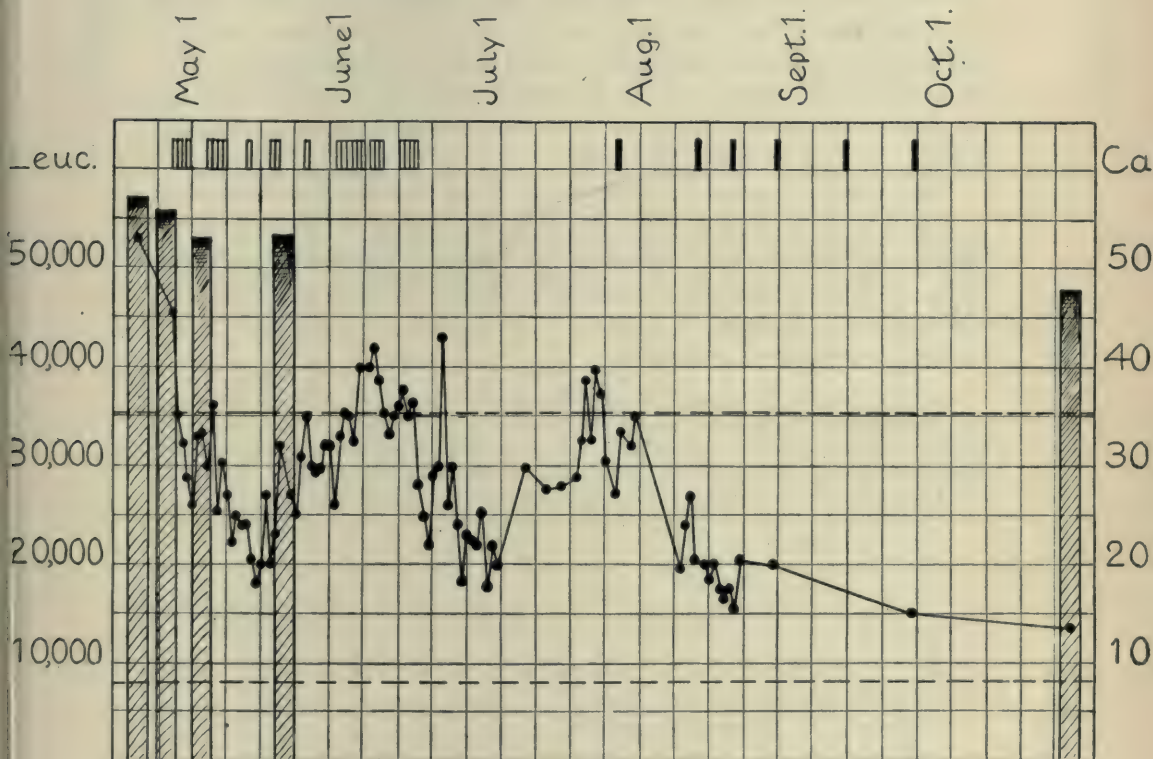


Fig. 3.—Chart showing the basal metabolism, shaded columns, and the leukocyte count, black dots and lines. The hollow rectangles at the top indicate Roentgen treatments, and the black ones radium treatments. The perpendicular rulings indicate periods of one week. The upper broken line represents the average basal metabolism for men from 50 to 60 years of age; the lower, the normal leukocyte count.

were obtained only while the patient was in the Sage metabolism ward, so that all the figures given in Table 3 were obtained before the irradiation was begun.

In Figure 3 the leukocyte count and the basal metabolism are compared. It will be seen that both of these were falling before the irradiation was begun. A slight fall in the basal metabolism occurred five days after the beginning of irradiation but was no greater than



had previously occurred spontaneously, and after twenty-three days, during which ten treatments were given, it had shown no further decrease. The Roentgen ray had no appreciable effect on the basal metabolism in this case. From the appearance of the leukocyte curve it looks as though a drop had been caused in the first few days. Prolonged treatment, moreover, entirely failed to produce an aleukemic blood picture.

The effect of the radium treatment at St. Luke's Hospital is rather more striking. The leukocyte curve shows a much more consistent fall after the radium exposures than after the Roentgen therapy. With this drop in the leukocytes the basal metabolism fell to 47.7 calories per square meter per hour, which is 36 per cent. above the normal average for men of the patient's age.

The results obtained on Anthony W. have recently been confirmed by a patient with myelogenous leukemia studied in the Massachusetts General Hospital in the service of Dr. Edsall. A preliminary report of the findings is given below. The basal metabolism was determined with the small Benedict Universal Respiration Apparatus, using the method of indirect calorimetry.

Dr. J. G., aged 51, height 171 cm. Six months previous to examination it was noticed that the patient's color was not good. For the previous six weeks he had suffered from weakness and lassitude. His blood picture was that of myelogenous leukemia and the spleen was enormously enlarged.

November 9 he felt much better. The size of the spleen was unchanged.

TABLE 4.—CLINICAL DATA IN CASE OF DR. J. G.

Date	Weight, Kg.	Leukocytes	Basal Metabolism Cals. per Sq. M. per Hr. (Linear Formula)
10/12/16	83.0	296,000	65.3
10/16/16	Radium treatment		
10/17/16	83.0	240,000	60.7
11/ 9/16	81.5	73,000	51.0

#### DIRECT AND INDIRECT CALORIMETRY

The agreement between the two independent methods of direct and indirect calorimetry in the case of Anthony W. is unusually close. The figure for total calories as measured by the former method is 1,054.0; by the latter method, 1,049.4. This indicates that there is no profound and unsuspected abnormality in the intermediary metabolism.

The water elimination was unusually high, 53 gm. an hour. He lost on an average of 29.5 per cent. of his calories in the vaporization of water under the atmospheric conditions in which normal men lose only 24.1 per cent. in this manner.

## SUMMARY AND CONCLUSIONS

A man with chronic lymphatic leukemia was studied five times in the respiration calorimeter. When first observed his metabolism was 44 per cent. above the average normal, falling a little with rest in bed. Intensive treatment with Roentgen rays caused a drop in the leukocyte count but did not appreciably affect the level of his metabolism. The water elimination through skin and respiratory passages was unusually high. Direct and indirect calorimetry gave total results which were almost identical, and no abnormal respiratory quotients were found.

After treatment with radium, a further and very marked fall occurred in the leukocyte count and, at the same time, a slight fall occurred in the basal metabolism.<sup>40</sup>

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40. The writers wish to thank Dr. Alexander Lambert for his courtesy in transferring the patient to the metabolism ward; Dr. Montgomery and Dr. Witherbee of the Rockefeller Hospital for the data of Roentgen-ray treatments; and Dr. Karl M. Vogel of St. Luke's Hospital for the data of radium treatments.

# CLINICAL CALORIMETRY

## TWENTY-FOURTH PAPER

### METABOLISM IN THREE UNUSUAL CASES OF DIABETES \*

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WITH THE TECHNICAL ASSISTANCE OF G. F. SODERSTROM

NEW YORK

The treatment of diabetes by the method of prolonged fasting has been conspicuously successful in recent years. The present paper deals with the measurement of the respiratory exchange and the total heat production of three patients, and supplements Paper 17 of this series by Allen and Du Bois,<sup>1</sup> which contains a review of the literature.

The patients were:

1. A diabetic woman who had been fasted to a state of emaciation, and who had a low tolerance for carbohydrate.

2. A diabetic man who had had the disease in a mild form, who showed a tolerance for 40 gm. of carbohydrate, but who was made completely diabetic ( $D:N=3.84$ ) on the second day after the administration of a diet containing considerable quantities of protein and fat. Subsequently, virtual starvation led within sixty hours to the disappearance of the sugar from the urine.

3. A diabetic man who at first manifested resistance to the influence of fasting, whose metabolism indicated complete diabetes, in that he had a  $D:N$  ratio of 3.97 when given meat and fat, and also eliminated that quantity of beta-oxybutyric acid theoretically derivable from the fat metabolism of the time. This patient subsequently developed a tolerance for 250 gm. of carbohydrate.

#### METHODS

The methods and calculations used in these observations were the same as those in the cases previously reported from this laboratory by Allen and Du Bois. The respiratory quotients and total metabolism were determined by the method described in the previous papers on

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\* From the Russell Sage Institute of Pathology, in affiliation with the Second Medical Division, Bellevue Hospital.

1. Allen, F. M., and Du Bois, E. F.: Metabolism and Treatment in Diabetes, *THE ARCHIVES INT. MED.*, 1916, **17**, 1010.



clinical calorimetry,<sup>2</sup> and, unless otherwise stated, were all basal observations; that is, they were made at least fifteen to eighteen hours after the last ingestion of food. Good alcohol check observations, made just before and after the observations on Cyril K., and at about the same time as the studies of the other two cases reported, assured the accuracy of the findings. The calculations involved in the determination of the protein respiratory quotient, complicated by glucose excretion from protein, were thoroughly discussed in Paper 17 of this series.<sup>3</sup>

Urine: The urinary examinations were made for total nitrogen by the Kjeldahl method; for ammonia, by the Folin method; for urinary sugar, by the Benedict method; for acetone bodies, by Shaffer's method; beta-oxybutyric acid, by Shaffer's method; sodium chlorid, by the Volhard method.

Blood: Blood sugar was determined by the method of Lewis and Benedict; nonprotein nitrogen, by Bock and Benedict's modification of Folin's method; urea, by the method of Van Slyke and Cullen; blood fat, by Bloor's method. The carbon dioxid combining power of the plasma was determined by the method of Van Slyke; alveolar air determinations were made by the Plesch-Higgins method.

The dextrose to nitrogen ratios are calculated as follows: The carbohydrate value in the food ingested is subtracted from the total glucose found in the urine. The remaining glucose excretion, divided by the total nitrogen excreted during the same period, gives the dextrose to nitrogen value. Its usual maximum value, as first shown by Mandel and Lusk,<sup>4</sup> is 3.65.

#### ROUTINE

The two male patients were kept in the Sage metabolism ward at Bellevue Hospital under the routine observation described in Paper 3 of this series.<sup>5</sup>

The one female patient was left in the open ward for one day under constant observation of the metabolism nurses. Every attempt was

2. Riche, J. A., and Soderstrom, G. F.: Clinical Calorimetry, Papers 2, 3 and 4, The Respiration Calorimeter of the Russell Sage Institute of Pathology in Bellevue Hospital; Gephart, F. C., and Du Bois, E. F.: The Organization of a Small Metabolism Ward; The Determination of the Basal Metabolism of Normal Men and the Effect of Food, *THE ARCHIVES INT. MED.*, 1915, **15**, 805-867.

3. Allen, F. M., and Du Bois, E. F.: Loc. cit., Note 1, p. 1018.

4. Mandel, A. R., and Lusk, G.: Stoffwechselbeobachtungen an einem Falle von Diabetes mellitus, mit besonderer Berücksichtigung der Prognose, *Deutsch. Arch. f. klin. Med.*, 1904, **81**, 472.

5. Gephart, F. C., and Du Bois, E. F.: Clinical Calorimetry, Paper 3, The Organization of a Small Metabolism Ward, *THE ARCHIVES INT. MED.*, 1915, **15**, 829.

made to have all diets administered as accurately as possible, and composed of simple foodstuffs of known composition. The careful collection of urinary samples, by separate bottles for each voiding, has been described.

#### REPORT OF CASES

CASE 1.—*History*.—Anna H., aged 31, married, born in United States. Occupation, telephone operator. Weight 32.5 kg.; height 169 cm.

The patient was transferred for one day to Bellevue from the Rockefeller Hospital, where she had been under the care of Dr. Fitz.

Family history and physical history were not remarkable.

She was married three and a half years prior to admission; she had had two miscarriages; health was good until June, 1915. She weighed at that time 145 pounds (66 kg.) with her clothes; then marked polydipsia and polyuria developed, with loss of weight and strength. July 4, 1915, sugar was found in her urine. August 5, a fast of three days made her sugar-free but in September she again had glycosuria.

October 16 she entered the Rockefeller Hospital and remained there five months. She weighed on entrance 49 kg. After fasting six days she became sugar-free, but in order to keep her so she was given a very low diet. For the two months previous to this observation her average total intake was 570 calories a day, made up of whisky (70 to 150 c.c.) and an average of 26 gm. of protein, 17 gm. of fat, and no carbohydrate. This food was taken mostly in the form of eggs. The day previous to the calorimeter determination she received whisky, 30 c.c.; protein, 30.9 gm.; fat, 23.4 gm.; carbohydrate, 2.5 gm., containing in all 458 calories.

The physical examination was negative save for extreme emaciation. Knee jerks were not obtained.

She went into the calorimeter March 31, 1916. During the next few months her tolerance remained low, she lost weight steadily and died July 18, 1916. Permission for necropsy was refused.

*Summary*.—A case of diabetes of nine months' duration in a young woman. Her sugar tolerance is very low. She can take two to six eggs a day and 100 c.c. of whiskey and remain sugar-free, but if more food is given, sugar appears in the urine. For five months she has been on a very low calory diet at the Rockefeller Hospital (see the foregoing) and her urine has been free from sugar.

I. Her total metabolism is the lowest recorded in the literature, being only 23.2 calories per square meter per hour, which is 37 per cent. below the average basal normal in women. Since her original weight with clothes had been 66 kg., one may be permitted to assume a weight of 62 kg. without clothes. Had her metabolism been normal for this weight, it would have been 63.3 calories per hour instead of 29.4 calories, which were actually measured when her weight had fallen to 32.5 kg. The extreme emaciation which had resulted in a reduction of body weight to nearly half of what it was originally, reduced the metabolism so low that only 40 per cent. of the original heat production was necessary for life. This reduction in metabolism is one of the theoretical benefits of the Allen fasting treatment.



II. The nitrogen excretion in the urine (0.39 gm. per hour) is the quantity commonly found in normal people. The total metabolism, however, is so low that the percentage of calories from protein is quite high, 35 per cent. (15 per cent. being the average normal).

III. The respiratory quotients average 0.82, a normal figure. From this, one may calculate that fat gives 39 per cent. of the calories of metabolism and carbohydrate 26 per cent. This corresponds to the utilization of 44 gm. of carbohydrate daily, and, since none was given in the food, these results are difficult to interpret.

CASE 2 (Table 1).—*History*.—The case of Joseph D. is unusual because of the sudden change from a rather mild diabetes to one of the greatest severity, followed by rapid recovery of sugar tolerance.

Joseph D., aged 33, single, born in Russia. Occupation, carpenter. Admitted to Bellevue Hospital Nov. 9, 1915; discharged Nov. 29, 1915.

The patient was always well until six years prior to admission, when he had a chancre. He took four to five glasses of beer a day and sometimes smoked thirty or forty cigarets a day. His weight three years prior to admission was 89.1 kg.; now it is 56.8 kg.

Three years prior to admission, without known cause, he became very thirsty, hungry and weak. Two years previously sugar was found in the urine. He was given a diet with carbohydrate much reduced in quantity, and also sodium bicarbonate. His urine remained free from sugar for over a year, when he gave up dieting and glycosuria recurred. Then his former symptoms returned; his eyesight had been rapidly failing, so that he had difficulty in reading. For the previous three months he had been at Gouverneur, Bellevue and City Hospitals. He felt well save for weakness and hunger.

*Physical Examination*.—Emaciated, poorly developed, hollow chested man; face flushed and slightly cyanotic; breath smelled of acetone; tongue deep red and papillae prominent; throat and tonsils deep red and glazed. There was marked clubbing of fingers.

*Lungs*: Percussion showed dulness at both apices, particularly behind, with narrowing of both apical isthmuses; the breath sounds here were rather sharp and high pitched; voice sounds slightly nasal and tactile fremitus diminished; no râles heard.

*Reflexes*: Neither knee jerks nor ankle jerks could be obtained.

*Extremities*: From the knee down there was dulling of sensation for touch, poor differentiation of sharp and dull pressure, and of heat and cold. This was most marked on the inner side of the lower legs.

Wassermann reaction positive.

Blood pressure: systolic, 90 mm.; diastolic, 75 mm.

When transferred to Bellevue Hospital the patient was placed in the metabolism ward. Here his diet was changed from a very liberal allowance to one containing only 1,500 calories, with 40 to 50 gm. of carbohydrate and 15 gm. of nitrogen. With this intake his sugar excretion fell in five days from 72 gm. a day to 22 gm., and the morning urine of the sixth day (November 15) was free from sugar. This led us to believe that the case was comparatively mild, and our opinion was checked by a calorimeter observation. The respiratory quotient was 0.79, a normal value, indicating carbohydrate oxidation.

The patient complained bitterly of low calorie diets, so it was decided to give large quantities of meat and fat. After the calorimeter observation on November 15 he was therefore given a diet containing 3,000 calories, with 25 gm. of protein nitrogen and only 3 gm. of carbohydrate. The diet pleased the patient, but in the following fifteen hours, with 3 gm. carbohydrate in the food, he excreted 25.8 gm. of glucose and 14.9 gm. of nitrogen. The D: N ratio



TABLE 1.—SHOWING SUDDEN CHANGE

Name and Date	Temperature		Food			Food N., Gm.	Urine N., Gm.	Excreta N., <sup>1</sup> Gm.	Nitrogen Balance Gm.
	Max.	Min.	Total Calories	Carbohyd., Gm.	Fat, Gm.				
Joseph D. 11/10/16	99.2	98.8	1,521	48	104	14.0	14.6	16.0	-2.0
11/11/16	98.6	97.8	1,495	42	102	14.6	16.8	18.3	-3.7
11/12/16	98.8	97.0	1,576	41	102	15.0	17.8	19.3	-4.3
11/13/16	98.8	97.6	1,724	41	104	14.8	16.8	18.3	-3.5
11/14/16	98.4	98.0	1,580	37	97	13.3	15.9	17.2	-3.9
11/15/16	98.8	98.0	3,067	3	261	24.6	17.9	20.4	+4.4
11/16/16	99.6	98.4	3,135	3	266	25.2	22.8	25.3	-0.1
11/17/16	100.4	98.8	1,849	2	133	12.7	12.3 <sup>3</sup>	13.6	-0.8
11/18/16	99.8	99.6	558	?	7	0.5	10.0 <sup>4</sup>	10.1	-0.6
11/19/16	98.8	98.0	567	3 <sup>2</sup>	17	4.8	9.6 <sup>5</sup>	10.1	-5.3
11/20/16	98.8	98.8	674	2	17	3.7	8.5 <sup>6</sup>	8.9	-5.2
11/21/16	98.0	97.8	1,091	2	57	4.5	11.2	11.7	-7.1
11/22/16	98.2	97.0	1,318	2	76	6.3	11.9	12.5	-6.2
11/23/16	97.0	97.0	1,527	25	85	7.8	11.5 <sup>7</sup>	12.3	-4.5
11/24/16	98.0	97.8	1,615	38	88	9.5	12.1 <sup>8</sup>	13.1	-3.6
11/25/16	99.2	97.4	2,120	50	132	12.6	12.3 <sup>9</sup>	13.6	-1.0
11/26/16	99.2	98.4	2,059	70	122	12.5	10.6 <sup>10</sup>	11.9	+0.7
11/27/16	99.0	98.0	753	22	27	3.9	6.9 <sup>11</sup>	7.3	-3.3
11/28/16	98.8	98.0	2,095	56	124	12.3	8.8	10.0	+2.3

1. Excreta nitrogen calculated as urine nitrogen plus 10 per cent. of food nitrogen.

2. Bread crumbs found in bed.

3. 16 hours 55 minutes.

4. 29 hours 35 minutes.

5. 25 hours.

6. 24 hours 30 minutes.

was 1.53. The next day, November 16, the patient felt very well and was allowed to sit in a chair. The diet was continued. The urine of this day showed 90.6 gm. of glucose and a complete D:N ratio, 3.84.

November 17 the diet was reduced to 1,850 calories containing 12.7 gm. of nitrogen and but 2 gm. of carbohydrate. Even with this diet the patient excreted 40.4 gm. of glucose and had a D:N ratio of 3.09. During the day the patient had abdominal pain and marked diarrhea, with mucus and some blood. Toward evening he complained of pain in the left jaw. Six hours after his last meal, which contained 65 gm. of fat and 7 gm. of protein nitrogen, a calorimeter observation was made and the respiratory quotient was found to be 0.714. This value is low, and the calculations show that he was oxidizing almost no carbohydrate. The D:N ratio of 2.53 confirmed the low respiratory quotient.

November 18 the patient was given a very low diet, only 550 calories, mostly in the form of alcohol, with less than 1 gm. of carbohydrate or protein. However, he managed to steal a slice of bread, so that an accurate D:N ratio for the day cannot be given. If one approximately estimates the weight of a slice of bread to be 40 gm., however, the D:N ratio would have been about 2.

A large alveolar abscess was discovered and opened and 2 or 3 c.c. of pus pressed out. The diarrhea stopped and the patient felt well and hungry.

## FROM MILD TO SEVERE DIABETES

Body Weight, Kg.	Urine Volume, C.c.	Calories per Kg.	Blood Pressure, Syst. Diast.	Urine Glucose, Gm.	NH <sub>3</sub> N, Gm.	Alcohol, Cals.	Sodium Bicarb., Gm.	D : N Ratio	Acetone plus Diacetic Acid
45.7	1,420	33	.....	71.97	1.295	...	20		
46.3	1,940	33	.....	45.09	0.558	...	20		
46.3	2,040	34	.....	37.45	0.628	80	15		
46.9	3,220	37	.....	32.90	0.584	214	15	....	0.65
47.1	3,420	34	.....	22.20	0.579	187	20	0	0.43
46.0	4,040	..	90-75	25.80	0.707	...	15	1.27	1.77
45.5	3,900	69	95-75	90.57	3.143	...	20	3.84	2.44
44.4	1,392	42	.....	40.38 <sup>3</sup>	2.220 <sup>3</sup>	281	30	3.09	2.02
44.0	1,060	13	95-75	41.33 <sup>4</sup>	1.130 <sup>4</sup>	482	25	2.0 (?)	0.58
45.6	3,880	12	.....	2.74 <sup>5</sup>	0.585 <sup>5</sup>	...	20	1.17 <sup>12</sup>	
44.7	3,760	17	95-75	0.00 <sup>3</sup>	0.264 <sup>6</sup>	441	30		
....	3,880	..	.....	0.00	0.304	441	18		
44.5	3,340	29	.....	0.00	.....	441	10		
44.4	3,400	34	100-80	0.00	.....	441	12		
....	3,620	..	.....	0.00	.....	402			
43.9	3,180	46	.....	0.00	.....	361			
44.7	3,600	42	.....	Trace	.....	321			
....	4,400	..	.....	Sl. trace	.....	321			
....	2,840	..	.....	0.00	.....	401			

7. 25 hours.

8. 23 hours 20 minutes.

9. 23 hours 40 minutes.

10. 24 hours 55 minutes.

11. 23 hours 5 minutes.

12. Eight-hour specimen.

November 19, the respiratory quotient was again nearly normal, 0.775, and by noon the urine was free from sugar.

November 23, the patient was put on a mixed diet with daily increasing carbohydrates.

November 26, with 70 gm. of carbohydrate in the food, there was a faint trace of glucose in the urine.

November 27, low diet day.

November 28, mixed diet containing 50 gm. of carbohydrate. The patient was returned to the hospital from which he had come.

It was difficult to keep this patient on a restricted diet except under constant supervision. This could be managed in the metabolism ward, but not in the open ward.

On further treatment he did not improve, and Jan. 4, 1916, he died in diabetic coma.

*Summary.*—This case shows very clearly how rapid may be the change in tolerance for carbohydrate in diabetes. The urine of the patient had become free from sugar when given a mixed diet con-

taining 40 gm. of carbohydrate, but the diet high in meat and fat, and alveolar abscess with the diarrhea, were sufficient to make him a complete diabetic; that is, with the theoretical D:N ratio continuing for one day. This is shown in Table 2.

TABLE 2.—ILLUSTRATING RAPID CHANGE IN CARBOHYDRATE TOLERANCE

Joseph D., a diabetic whose urine was free from sugar, became completely diabetic (D:N=3.84) sixteen hours after the ingestion of meat and fat in large quantity, and, on reducing the diet, became free from sugar in about sixty hours.

Date	Day	Diet	Nitrogen in Urine per Hour	D:N	R. Q.	Calories per Sq. M. per Hour	Variation from Normal in Per Cent.
1915 11/16	1	Basal, then high protein...	0.385	....	0.79	37.9	— 5
11/16	2	High protein.....	.....	3.84			
11/17	3	Less protein.....	0.356	2.53	0.714	50.7*	+28*
11/18	4	Fasting and stolen bread					
11/19	5	Fasting and stolen bread..	0.330	0.0	0.78	38.6	— 3

\* Restless during both hours while in the calorimeter. Had pain during the last hour and asked to be taken out.

Accompanying this absolute inability to metabolize glucose, there developed at the same time some inability to metabolize fat to its normal end-products: Unfortunately, the only figures we have to report are the values of urinary ammonia and of acetone and diacetic acid excretion. The ammonia values suggest that the formation of acetone bodies was only in moderate quantity.

When the complicating factors were eliminated, the patient rapidly improved and again tolerated a mixed diet of 2,000 calories, containing 50 gm. of carbohydrate.

While the D:N ratio may be altered by the ingestion of carbohydrate food, the respiratory quotient quickly reveals if carbohydrate is being metabolized. The validity of the urinary D:N=2.53 on November 17 is checked excellently by the calorimeter observations of the same date which showed a very marked drop in the respiratory quotient to 0.714, indicating that very little glucose was being oxidized. Hence, on this day no carbohydrate could have been surreptitiously taken by the patient.

The patient was restless while in the calorimeter on the day of diabetes, so that the increase of 28 per cent. in his metabolism is not to be accepted without qualification. The hourly urinary nitrogen was the same during all three calorimeter periods. It is possible that the



effect of the stimulus due to the high protein metabolism of Day 2 outlasted the presence of the stimulus itself.<sup>6</sup>

CASE 3<sup>7</sup> (Tables 3, 4 and 5).—*History*.—Cyril K., aged 19, single, college student, born in the United States.

The patient was admitted to the Presbyterian Hospital Dec. 7, 1915; transferred to Bellevue Hospital Dec. 14, 1915; retransferred to Presbyterian Hospital Dec. 23, 1915; discharged March 7, 1916.

The family history was negative. The patient's health was always good. Fourteen months prior to admission he passed a life insurance examination. There had been no recent infection. October 19 he weighed 78.2 kg. with his clothes on and was then feeling perfectly well. Early in November he began to lose weight, felt weak, tired, without ambition. November 15 a physician found 4.5 per cent. sugar in the urine. He was given a diet containing much meat, whole wheat bread and buttermilk. He had polydipsia and polyuria. About November 20 he weighed 70.5 kg. with his overcoat on. December 1 he felt drowsy and slept a great deal. December 4 he felt very weak, nauseated and vomited, the drowsiness continuing. His last food was December 6 at 7 p. m.

December 7 he entered Presbyterian Hospital. Hemoglobin, 80 per cent.; white cells, 15,500; Wassermann negative.

Under Dr. Geyelin's care he was fasted until December 11. He seemed to be getting more drowsy; respirations were of the air hunger type; he was nauseated. Each day he received 53 to 114 gm. soda bicarbonate, most of it intravenously.

December 11 he was given food, as fasting did not seem to improve his condition. He received mostly milk and eggs. December 13 his condition improved. He was less drowsy and there was less air hunger.

December 14, transferred to Bellevue Hospital.

*Physical Examination*.—The patient was 6 feet 2 inches tall; of large frame; very thin, but not yet emaciated, with flushed face, pinched nose, drooping eyelids. He was slightly drowsy, but mentally clear. There was a marked acetone odor to the breath. Respirations were deep, 18 to the minute. The mucous membrane, gums and tongue were bright red and glazed. There were small furuncles on the right pinna and two on the left side of the neck. The remainder of the physical examination was not remarkable.

December 15. Put in the calorimeter this morning after a milk diet.

December 16. The patient read the newspaper; no nausea, said he felt very much better than two days previously. Clinically, he was much improved, although the laboratory findings did not indicate much change. He was placed in the calorimeter after a day of protein food.

December 18. Basal calorimeter observation this morning. Abscess on back of neck incised and 2 c.c. of greenish pus evacuated. Started fasting.

December 19. Abscess dressed; 2 c.c. pus obtained. Blood examination showed: white cells, 10,200; red cells, 5,746,000.

December 20. Basal calorimeter observation.

December 21. Still fasting; patient improving steadily; breathing normal; tongue clean; patient not at all drowsy, but reading all day; abscess still discharging small amount of pus.

6. Lusk, G.: Animal Calorimetry, Paper 11. An Investigation Into the Causes of the Specific Dynamic Action of the Foodstuffs, Jour. Biol. Chem., 1915, **20**, 569.

7. A preliminary report of this case has been given by Geyelin and Du Bois: A Case of Diabetes of Maximum Severity with Marked Improvement, Jour. Am. Med. Assn., 1916, **66**, 1532. Further details of the clinical and laboratory data will be published shortly by Geyelin.

TABLE 3.—CLINICAL DATA IN—

Name and Date	Body Weight, Kg.	Total Calories	Calories per Kg.	Food		Food N, Gm.	Fluid Intake, C.c.	NaCl Intake, Gm.	Sodium Bicarb., Gm.	Chalk, Gm.	Temperature	
				Carbohyd., Gm.	Fat, Gm.						Max.	Min.
Cyril K. 12/14/15	....	986	..	53.0	51.0	9.8	3,430	8.00+	25	15	Normal	
12/15/15	56.7	934	12	24.0	41.0	19.0	5,850	18.00	50	20	Normal	
12/16/15	56.8	458	9	0.4	5.6	16.2	4,525	19.93	55	20	Normal	
12/17/15	58.0	191	3	0.4	2.9	6.3	6,560	27.29	60	30	100.8	98.6
12/18/15	56.5	0	0	Fast day		....	4,250	10.00	45	20	98.4	97.6
12/19/15	57.2	0	0	Fast day		1.3	5,550	7.10	55	25	Normal	
12/20/15	56.9	60	1	6.0	0.3	2.6	3,850	8.60	30	20	Normal	
12/21/15	56.6	83	1	6.6	0.3	3.2	4,150	12.12	5	5	Normal	
12/22/15	54.3	108	2	11.4	0.4	3.4	4,060	17.19	..	..	98.2	97.4
12/23/15	....	...	..	....	....	....	....	....	..	..	.....	
2/15/16	....	...	..	252.0	6.0	9.5	....	....	..	..	.....	
2/16/16	....	...	..	270.0	10.9	7.0	....	....	..	..	.....	

\* Exereta nitrogen calculated as urine nitrogen plus 10 per cent. of food nitrogen.

† Thirteen hours and forty-five minutes.

‡ Nonprotein nitrogen, 24.7 mg.; blood urea, 9.1 mg.; blood fat, 0.49 per cent.

TABLE 4.—

Subject	Age	Date	Height, Cm.	Weight, Kg.	Surface Area, Linear	Total Calories per Hour	Calories per Sq. M. per Hour
Case 3 (Cyril K.)....	19	12/15/15	187	56.7	1.79	81.9	45.7
		12/16/15	...	56.8	1.79	76.4	42.6
		12/18/15	...	56.5	1.79	73.2	40.8
		12/20/15	...	56.9	1.79	66.3	37.0
		12/22/15	...	54.3	1.75	62.8	35.9
		2/16/16	...	45.8	1.66	43.0	25.9
		3/ 8/16	189	48.3	1.72	50.0	29.1
Case 2 (Joseph D.)	33	11/15/15	170	45.5	1.48	56.1	37.9
		11/17/15	...	44.4	1.47	74.5	50.7
		11/19/15	...	45.6	1.48	57.1	38.6
Case 1 (Anna H.)...	31	3/31/16	169	32.5	1.27	29.5	23.2

\* D:N during calorimeter period.

## —A CASE OF DIABETES

Blood Pressure Syst. Dias.	Urine Vol., C.c.	Urine* N., Gm.	Nitrogen Balance, Gm.	Urine Glucose, Gm.	D:N Ratio	Urine NaCl, Gm.	NH <sub>3</sub> N., Gm.	Acetone + Diacetic Acid Gm.	Beta- oxy- butyric, Gm.	Alveo- lar CO <sub>2</sub> Ten- sion, Mm. Hg	Blood	
											CO <sub>2</sub> Vol. %	Glucose, Mg.
.....	4,715	37.7	-28.9	118.5	....	.....	5.41	3.78†	55.2	....	....	.....
118-78	5,290	36.4	-19.3	167.9	3.97	13.21	5.214	5.61	70.9	....	29.2	.....
114-64	5,710	38.3	-23.7	153.4	4.01	16.37	3.537	4.99	75.1	{21.0 15.1	28.4	.....
114-78	6,900	36.3	-30.6	140.3	3.87	22.85	3.818	.....	87.4	23.4	51.3	.....
108-60	3,980	20.0	-20.1	55.1	2.76	16.83	2.914	.....	58.5	22.9	52.0	0.150
102-70	4,700	16.7	-15.5	44.3	2.65	15.43	2.290	.....	56.8	30.6	73.9	.....
.....	4,080	14.1	-11.8	35.3	2.44	13.44	1.603	.....	41.2	33.7	75.8	0.177
104-64	6,020	14.4	-11.5	39.7	2.65	19.86	1.524	.....	26.2	....	....	0.170†
104-70	3,770	18.3	-15.2	26.0	1.12	13.21	1.429	.....	11.0	{35.3 31.0	78.2	0.181
.....	.....	.....	.....	.....	....	.....	.....	.....	.....	....	72.1	0.206
.....	.....	.....	.....	0.0								
.....	.....	.....	.....	Trace								

## —SUMMARY CHART—DIABETES

Variation from Average Normal, Per Cent.	R. Q.	Nonprotein R. Q.	D : N Ratio*	Per Cent. Calories from			Remarks
				Protein	Fat	Carbohyd.	
+15	0.687	0.609	5.05	21	79	0	After breakfast
+ 7	0.714	0.743	3.65	25	75	0	After breakfast
+ 3	0.707	0.706	2.63	21	79	0	Basal
- 7	0.721	0.718	2.21	15	82	3 (?)	Basal
-10	0.734	0.728	1.53	20	74	6	Basal
-35	0.915	0.970	0.0	31	7	62	Basal
-27	0.860	0.872	0.0	16	37	47	Basal
- 5	0.790	0.787	0.0	17	60	23	Basal
+28	0.714	0.711	2.53	8	90	2 (?)	After dinner
- 3	0.775	0.769	0.0	16	66	18	Basal
-37	0.815	0.822	0.0	35	39	26	Basal



TABLE 5.—DATA OF CALORIMETER—

Subject, Date, Weight, Surface Area, Linear Formula	Period	End of Period	Carbon Dioxid, Gm.	Oxygen, Gm.	R. Q	Water, Gm.	Urine N per Hour, Gm.	Indirect Calo- rimetry, Cal.	Heat Elimi- nated, Cal.
Case 2 (Joseph D.) 11/15/15 45.5 Kg. 1.48 Sq. M.	Prelim.	11:39 a.m.	....	....	....	....	....	....	....
	1	12:39	18.2	17.3	0.768	28.7	0.385	56.8	53.4
	2	1:39	18.6	16.7	0.812	27.8	0.385	55.4	58.0
Joseph D. .... 11/17/16 44.4 Kg. 1.47 Sq. M.	Prelim.	6:04 p.m.	....	....	....	....	....	....	....
	1	7:04	22.6	22.5	0.730	24.1	0.356	73.6	68.1
	2	7:56	19.3	20.1	0.698	23.7	0.356	65.4	59.8
	Aver.	....	....	....	....	....	....	....	....
Joseph D. .... 11/19/15 45.6 Kg. 1.48 Sq. M.	Prelim.	11:43	....	....	....	....	....	....	....
	1	12:43	18.9	17.6	0.780	34.5	0.330	58.2	64.7
	2	1:43	18.0	17.0	0.770	34.1	0.330	56.1	60.2
	Aver.	....	....	....	....	....	....	....	....
Case 3 (Cyril K.).. 12/15/15 53.7 Kg. 1.79 Sq. M.	Prelim.	12:31	....	....	....	....	....	....	....
	1	1:31	23.3	23.6	0.718	31.1	1.34	....	80.1
	2	2:31	25.1	27.3	0.656	36.4	1.34	....	89.5
	Aver.	....	....	....	....	....	....	81.9*	....
Cyril K. .... 12/16/15 56.8 Kg. 1.79 Sq. M.	Prelim.	12:31	....	....	....	....	....	....	....
	1	1:31	24.1	24.5	0.715	32.1	1.44	77.8†	93.6
	2	2:31	23.3	23.7	0.714	31.8	1.44	75.1†	85.9
	Aver.	....	....	....	....	....	....	....	....
Cyril K. .... 12/18/15 56.5 Kg. 1.79 Sq. M.	Prelim.	11:31	....	....	....	....	....	....	....
	1	12:31	22.7	22.3	0.740	31.9	0.927	....	86.0
	2	1:31	21.5	22.6	0.693	28.0	0.927	....	81.9
	3	2:31	22.2	23.5	0.688	29.5	0.927	....	85.1
	Aver.	....	....	....	0.707	....	....	73.2	....
Cyril K. .... 12/20/15 56.9 Kg. 1.79 Sq. M.	Prelim.	12:12	....	....	....	....	....	....	....
	1	1:12	20.2	20.2	0.728	20.3	0.533	....	72.5
	2	2:12	20.5	21.6	0.691	22.3	0.533	....	72.6
	3	3:12	19.9	19.5	0.742	20.6	0.533	....	67.3
	Aver.	....	....	....	0.720	....	....	66.4	....
Cyril K. .... 12/22/15 54.3 Kg. 1.75 Sq. M.	Prelim.	11:42	....	....	....	....	....	....	....
	1	12:42	19.7	19.6	0.731	21.4	0.60	63.6	64.2
	2	1:42	19.9	19.5	0.742	21.4	0.60	63.4	67.2
	3	2:42	19.0	19.0	0.729	20.8	0.60	61.5	64.5
	Aver.	....	....	....	....	....	....	62.8	....

\* Calculations based on assumed D:N ratio of 3.65.

† Calculations based on assumed nonprotein R. Q. of 0.70.

—EXPERIMENTS—DIABETICS

Direct Calorimetry, Cal.	Rectal Temp., C.	Average Pulse	Work-Adder, Cm.	Non-protein R. Q.	Per Cent. Calories from			Calories per Hour		Remarks
					Protein	Fat	Carbohyd.	Per Kg.	Per Sq. M.	
....	36.4	....	..	.....	..	..	..	....	....	Basal; diabetes
51.5	36.4	?	12	0.760	18	67	15	1.25	38.4	Asleep 45 min.
56.6	36.4	68	24	0.814	18	52	30	1.22	37.5	Somewhat rest- less; awake
....	37.5	96	..	.....	..	..	..	....	....	6 hrs. after dinner
77.8	37.8	99	32	0.732	..	..	..	....	....	Restless
59.5	37.8	103	31	0.697	..	..	..	....	....	Restless; 52 min. period
....	....	...	..	0.714½	8	89	3	1.68	50.7	½Aver. per hour
....	36.6	64	..	.....	..	..	..	....	....	Basal, second fast day
53.2	36.4	72	20	0.775	15	65	20	....	....	Reading 30 min.; asleep 10 min.
58.8	36.4	65	13	0.763	16	68	16	....	....	Quiet; asleep 30 min.
....	....	....	..	.....	..	..	..	1.25	38.6	{ At 6:30 a.m., ate protein 19, fat 18, carbo. 24
....	37.1	60	..	.....	..	..	..	....	....	
78.5	37.1	59	15	0.745	..	..	..	....	....	Quiet; slept 5 min.
82.0	37.0	67	32	0.661	..	..	..	....	....	Fairly quiet; awake
....	....	....	..	.....	21	79	0	1.44	45.7	{ Glucose in urine 6.76 gm. per hr.; D:N ratio 5.05
....	37.0	60	..	.....	..	..	..	....	....	{ At 6:30 a.m., pro- tein 26, fat 7, carbo. 0; sod. bicar. at 9 a.m.
86.8	36.9	65	20	0.743	24	76	0	1.37	43.4	Slightly restless; read 50 min.
75.5	36.8	62	15	0.743	25	75	0	1.32	41.9	Much quieter; awake; did not read
....	....	....	..	.....	..	..	..	....	....	{ Glucose in urine 5.24 gm. per hr.; D:N ratio 3.65
....	37.0	62	..	.....	..	..	..	....	....	{ Basal; first fast day; sod. bicar. at 9 a.m.
77.2	36.8	63	15	0.750	..	..	..	....	....	Reading quietly
75.6	36.7	61	8	0.689	..	..	..	....	....	Reading 15 min.; quiet
74.3	36.5	59	19	0.682	..	..	..	....	....	Fairly quiet
....	....	....	..	0.706	21	79	0	1.30	40.8	{ Glucose in urine 2.44 gm. per hr.; D:N ratio 2.63
....	36.3	....	..	.....	..	..	..	....	....	Basal; third fast day
68.0	36.2	57	20	.....	..	..	..	....	....	Quiet; reading
69.4	36.2	56	22	.....	..	..	..	....	....	Quiet; reading
65.5	36.2	57	14	.....	..	..	..	....	....	Very quiet
....	....	....	..	0.718	15	82	3	1.17	37.0	{ Urine glucose 1.18 gm. per hr.; D:N ratio 2.2
....	36.6	....	..	.....	..	..	..	....	....	{ Basal; fifth fast day
58.8	36.5	59	14	0.724	20	75	5	....	....	Reading; quiet
65.2	36.5	59	24	0.738	20	71	9	....	....	Reading 30 min.; slightly restless
68.1	36.6	59	12	0.721	20	76	4	....	....	Reading 30 min.; quiet
....	....	....	..	.....	..	..	..	1.16	35.9	{ Glucose in urine 0.92 gm. per hr.; D:N ratio 1.53

TABLE 5.—DATA OF CALORIMETER

Subject, Date, Weight, Surface Area, Linear Formula	Period	End of Period	Carbon Dioxid, Gm.	Oxygen, Gm.	R. Q.	Water, Gm.	Urine N per Hour, Fm.	Indirect Calo- rimetry, Cal.	Heat Elimi- nated, Cal.
Cyril K. .... 2/16/16 45.8 Kg. 1.66 Sq. M.	Prelim.	11:47	....	....	....	....	....	....	....
	1	12:47	15.8	11.9	0.967	13.7	....	....	45.0
	2	1:48	15.3	12.7	0.877	14.1	....	....	47.0
	3	2:47	16.8	13.5	0.901	16.7	....	....	48.4
	Aver.	....	....	....	....	....	0.506	43.0	....
Cyril K. .... 3/8/16 48.3 Kg. 1.72 Sq. M.	Prelim.	12:58	....	....	....	....	....	....	....
	1	1:58	17.6	14.8	0.866	20.9	0.301	49.9	50.9
	2	2:58	17.5	14.9	0.854	21.3	0.301	50.1	53.6
	Aver.	....	....	....	....	....	....	50.0	....
Case 1 (Anna H.) 3/31/16 32.5 Kg. 1.27 Sq. M.	Prelim.	11:52	....	....	....	....	....	....	....
	1	12:52	10.2	9.2	0.804	17.5	0.390	30.2	31.8
	2	1:52	9.5	8.2	0.840	15.6	0.390	27.2	29.5
	3	2:52	10.4	9.4	0.900	16.1	0.390	30.9	34.2
	Aver.	....	....	....	....	....	....	....	....

December 22. Basal calorimeter observation. Food given in small amounts; alkali medication discontinued.

December 23. Improving steadily.

December 25. Patient was returned to Presbyterian Hospital. In a week he became permanently sugar-free, and without acetone bodies in his urine.

February 15, 1916, he returned to Bellevue for a calorimeter observation. That day he received 252 gm. of carbohydrate, 60 gm. of protein and 69 gm. of fat without showing sugar in the urine.

February 16, after being in the calorimeter he was given 270 gm. of carbohydrate, 44 gm. of protein and 11 gm. of fat in two and one-half hours. After this large meal he showed only a trace of sugar in his urine.

February 27. A peritonsillar abscess was accompanied by marked lowering of the carbohydrate tolerance.

March 7. Carbohydrate tolerance regained; blood sugar normal, 0.08 per cent. Patient weighed 47.7 kg. He was taking a diet containing 2,500 calories.

March 8. Put in the calorimeter and then sent to his home. Later he developed pulmonary tuberculosis, with high fever.

In February, 1917, the patient was improving somewhat. He died March 17, 1917, having been free from urinary glucose and acetone bodies for 125 days.

The patient was an ideal subject for metabolism study. He was intelligent and anxious to cooperate in all the necessary procedures. He was very quiet when in the calorimeter. The element of increased restlessness frequently observed in disease and suggested by Magnus-Levy<sup>8</sup> as a cause of the increased metabolism sometimes recorded, was therefore absent in this individual.

8. Magnus-Levy, A.: Respirationsversuche an diabetischen Menschen, Ztschr. f. klin. Med., 1905, 56, 83.



## —EXPERIMENTS—DIABETICS—(Continued)

Direct Calorimetry, Cal.	Rectal Temp., C.	Average Pulse	Work-Adder, Cm.	Non-protein R. Q.	Per Cent. Calories from			Calories per Hour		Remarks
					Protein	Fat	Carbohyd.	Per Kg.	Per Sq. M.	
....	34.9	....	..	.....	..	..	..	....	....	Basal; bed
54.4	35.2	44	7	1.059	..	..	..	....	....	Very quiet; reading
58.8	35.4	44	9	0.914	..	..	..	....	....	61 min. period; very quiet
43.2	35.3	44	13	0.945	..	..	..	....	....	59 min. period; very quiet; reading 50 min.
....	....	....	..	0.970	31	7	62	0.94	25.9	No glucose in urine
....	36.6	....	..	.....	..	..	..	....	....	Basal
43.1	36.4	....	13	0.879	..	..	..	....	....	Quiet; reading 15 min.; sleeping 15 min.
43.5	36.2	57	9	0.864	..	..	..	....	....	Very quiet; reading whole hour
....	....	....	..	0.872	16	37	47	1.08	29.1	No glucose in urine
....	36.1	....	..	.....	..	..	..	....	....	Basal; in bed
25.7	35.9	60	6	0.805	34	44	22	....	....	Reading 45 min.
25.5	35.7	57	3	0.864	38	28	34	....	....	Very quiet; asleep 30 min.
37.8	35.9	59	16	0.797	33	46	21	....	....	Quiet; reading 10 min.
....	....	....	..	.....	35	39	26	0.91	23.2	

*Summary.*—This remarkable case may be reported in five successive periods, each sharply distinguished, and in a sixth period observed after an interval of two months. Table 6 represents the average daily figures obtained.

*I. First Fasting Period.*—The D:N in the urine during three days averaged 2.7 per day. The urinary nitrogen averaged 27.5 gm. daily, which is the highest level of nitrogen excretion ever recorded during fasting. It is threefold the quantity of nitrogen eliminated in ordinary fasting, and, therefore, brings this case of human diabetes in accord with the changes in protein metabolism noted in depancreatized and phlorhizinized dogs. During this period the blood-sugar was at its maximum (0.326 per cent.), beta-oxybutyric acid elimination reached 39.6 gm., and a low carbon dioxid tension in the blood betokened a considerable acidosis. Despite the administration, chiefly by intravenous injection, of between 53 and 114 gm. of sodium bicarbonate daily, the patient became increasingly drowsy, respirations were of the air hunger type, and he suffered from nausea.

*II. Mixed Diet Period.*—As fasting did not improve the patient's condition, he was given a diet of milk and eggs for four days, and on the third day his condition improved, he became less drowsy and there was less air hunger. After subtracting the carbohydrate ingested from the glucose eliminated in the urine, the D:N may be calculated as 1.9.

Although this indicates an oxidation of carbohydrate, the other metabolism factors of the pathologic complex show little improvement. Thus, the dietary contained 7.2 gm. of nitrogen daily and the urine 34.5 gm., an excess of 27.3 gm. above that contained in the food. The waste of 27.3 gm. of body nitrogen daily compares with a loss of 27.5 gm. daily during the fasting period immediately preceding. During this period of mixed diet the daily quantity of beta-oxybutyric acid eliminated increased to 56.5 gm. and the blood carbon dioxid tension fell to an average level of 22.1 mm. of mercury. It is difficult to find in the evidence presented by the metabolism data any cause for the improvement in the clinical condition.

TABLE 6.—METABOLISM OF CYRIL K.

Weight during Periods I-V, between 57.6 and 54.3 kg.; during Period IV, 45.8 kg.

Period	Date	Length of Period, Days	D:N	R. Q.	Urine		Blood		Calories per Sq. M. Surface	Variation from Normal, Per Cent.
					Nitrogen, Gm.	Beta-oxybutyric Acid, Gm.	Glucose in 100 C.c., Gm.	CO <sub>2</sub> Tension, Mm. Hg		
I. First fasting.....	1915 Dec. 8-10	3	2.7	.....	27.5	39.6	0.326	28.5		
II. Mixed diet.....	11-14	4	1.9	.....	34.5	56.5	0.312	22.1		
III. High protein-fat diet	15-17	3	3.9	0.687	37.0	77.8	.....	{19.6} {35.4}	44.1	+11
IV. Second fasting.....	18-21	4	2.7	0.714	16.3	45.7	0.166	44.4	38.9	- 2
V. Convalescence.....	22	1	1.1	0.734	18.3	10.9	0.181	52.8	35.9	-10
(Interval, 2 months)	1916 Feb.									
VI. Recovery.....	10	1	0.0	0.97	12.2	0.0	.....	.....	25.4	-36

*III. High Protein-Fat Diet Period.*—During a period of three days the patient was given a diet of protein and fat, and a D: N ratio of 3.9 was determined. Although no experimental data are available to show why this ratio is higher than 3.65, yet it is possible that the extra sugar may have been derived from a reduction in the quantity of sugar in the body, as would have been indicated by a lowering of the level of blood-sugar. The quantity of "extra sugar," or that eliminated in excess of a D: N ratio of 3.65, may be calculated as being 57 gm. during the three-day period. The patient weighed 57 kg., so that a fall of 0.1 per cent. in the quantity of blood sugar would explain the origin of the "extra sugar." The diet contained an average of 13.7 gm. of nitrogen daily, the urine 37.0 gm., a difference of 23.3 gm., or almost as great a loss of body nitrogen as during the two preceding periods. On the second day of this diet the patient manifested marked clinical improvement, sat up in bed and said he felt much better than two days

before, yet on this day the carbon dioxid tension in the blood fell to its lowest level of 19.6 mm. of mercury, and 75 gm. of beta-oxybutyric acid were eliminated. On the third day, however, the carbon dioxid tension reached 35.4 mm., a level which is more in accord with the actual improvement of the patient. During this period an average of 55 gm. of sodium bicarbonate were given daily.

The beta-oxybutyric acid excretion averaged 77.8 gm. On the two days in which calorimeter investigations were conducted the results may be computed to show how much of the total metabolism of fat reached beta-oxybutyric acid as its terminal stage. When fat is thus oxidized it is theoretically possible for 21.7 per cent. of the quantity of carbon dioxid ordinarily eliminated in the respiration to remain unoxidized in the form of beta-oxybutyric acid. Remembering this, one may make the computation shown in Table 7.

TABLE 7.—BETA-OXYBUTYRIC ACID ELIMINATION

The quantity of beta-oxybutyric acid eliminated per hour was slightly in excess of the quantity derivable from the fat metabolism of the time.

Date	Urine			Calories	CO <sub>2</sub> , Gm.	Nonprotein CO <sub>2</sub> , Gm.	Theoretical Beta-oxy- butyric Acid from Fat, Gm.
	Nitrogen, Gm.	D:N	Beta-oxy- butyric Acid, Gm.				
12/15	1.34*	3.97	3.13	81.9	24.2	18.9	3.09
12/16	1.44*	4.01	2.95	76.4	23.7	18.1	2.96

\* Eliminated per hour during calorimeter period.

It is apparent from Table 7 that on one of the two successive days the hourly elimination of beta-oxybutyric acid was slightly greater than the quantity derivable from the fat metabolism of the hours spent in the calorimeter. The slight excess may have been due to a slightly greater average of fat metabolism during the twenty-four-hour period than that observed during the calorimeter period, or it may have been due to an origin of beta-oxybutyric acid from those amino-acids which yield it. A considerable origin of beta-oxybutyric acid from the latter source is not indicated.

The picture presented during this period is one in which all the glucose derivable from protein and all the beta-oxybutyric acid formed as an end-product of the oxidation of fat is completely eliminated in the urine. Though Magnus-Levy<sup>9</sup> computed such results for a diabetic and comatose youth, the actual demonstration of this condition is here presented for the first time.

9. Magnus-Levy, A.: Die Acetonkörper, *Ergebn. d. inn. Med.*, 1908, **1**, 385.



*The Respiratory Quotient.*—An analysis of the diabetic respiratory quotient has already been presented in one of this series of papers.<sup>10</sup> During the period of a high protein-fat diet it is evident that the metabolism was highly abnormal. Only protein and fat were oxidized, and these only partially. It has been shown that when the D:N ratio is 3.65, the protein respiratory quotient becomes 0.632 instead of 0.80 normally, and when beta-oxybutyric acid is produced as the end-product of fat metabolism, the calculated respiratory quotient of fat falls to 0.669 instead of being 0.707, its normal value.

Were this the whole story, one should find agreement between the respiratory quotient as calculated from the end-products of metabolism during the period of severe diabetes and that actually found. Thus, on December 16 when the diabetes was complete, one might calculate the respiratory quotient as follows:

	Respired Gases		R. Q.
	O <sub>2</sub> Liters	CO <sub>2</sub> Liters	
If the D:N were 3.65, then 1.44 gm. N in urine =	4.58	2.91	0.63
If 18.1 gm. nonprotein CO <sub>2</sub> be derived from fat less unoxidized beta-oxybutyric acid,	13.77	9.21	0.67
	18.35	12.12	0.66

The respiratory quotient was actually 0.715, and, as a matter of fact, quotients as low as 0.66 have not been reported in trustworthy experiments on the basal metabolism in diabetes. It is evident that some accessory factors must come into play. It is highly probable that the interaction between acid formation and bicarbonate neutralization plays an important rôle in diabetes. Thus, if beta-oxybutyric acid formed from fat neutralizes sodium bicarbonate, the diabetic respiratory quotient for fat would be 0.715 instead of 0.669. Again, if the acid were neutralized with ammonia instead of with bicarbonate, it would have been at the expense of ammonia which normally would have combined with carbon dioxid in urea formation. The carbon dioxid would tend to be liberated in the respiration and the quotient would be increased.

Under certain conditions of recovery from the diabetic condition, respiratory quotients of 0.80 during fasting have been noted by Joslin,<sup>11</sup> and this has been attributed to the oxidation of beta-oxybutyric acid, which gives a respiratory quotient of 0.889. It should be noted, however, that if sodium beta-oxybutyrate be oxidized to bicarbonate of sodium, the respiratory quotient of the substance would be only 0.667. The unknowable interplay of factors which govern the finer details of

10. Lusk, G.: Clinical Calorimetry, Paper 8. On the Diabetic Respiratory Quotient, THE ARCHIVES INT. MED., 1915, **15**, 939.

11. Joslin, E. P.: Treatment of Diabetes Mellitus, New York, 1916, p. 96.

the respiratory quotient in diabetes prevents its precise interpretation. It is sufficient to bear in mind, however, that the quotient of 0.69, which is frequently found in total diabetes, indicates the oxidation of fat with the neutralization of sodium bicarbonate by the beta-oxybutyric acid formed. The following summary compares the respiratory quotients obtained under different conditions in this laboratory:

	D:N	R. Q.	Nonprotein R. Q.
Diabetic man, Cyril K.....	3.97	0.687	0.699
Diabetic man, Gerald S.....	3.50	0.697	0.700
Phlorhizinized dog .....	3.54	0.687	0.704

December 15 the nonprotein respiratory quotient of Cyril K. was 0.699, which is sufficiently close to 0.715 to indicate that the oxidation of fat proceeded in such a manner that some carbon dioxid was driven off from combined bicarbonate in the blood. December 16, under essentially the same condition of diabetes in its most intense form, the nonprotein respiratory quotient was 0.743. The alveolar tension of carbon dioxid was taken immediately before and immediately after the respiration experiment, and it fell from 0.21 mm. of mercury to 0.15 mm. This suggests an increased neutralization of acid by the blood with expulsion of carbon dioxid. It is this extra elimination of carbon dioxid which may play some part in this otherwise inexplicably high nonprotein respiratory quotient.

Grafe<sup>12</sup> calculated that if all the nitrogen of protein metabolism were eliminated as ammonia, the quotient would rise from the normal of 0.80 to 0.88. When, in our experiments, a correction of the respiratory quotient of protein was attempted because of the large elimination of ammonia, this has not increased the R. Q. more than 0.01.

*The total heat production* during this period of complete diabetes presents some interesting aspects. The method of calculating the heat produced from protein metabolism in severe diabetes, as described by Lusk,<sup>13</sup> has here been followed.

When the D:N ratio has fallen below 3.65, the effect on the respiratory quotient and the heat value of protein has been calculated by making deductions for the separate periods, remembering that each gram of glucose derived from protein would normally be associated with

Heat production .....	3.692 cal.
Oxygen absorption .....	1.067 gm.
Carbon dioxid elimination.....	1.467 gm.

It should be further remembered that when ammonia is converted into urea by union with carbon dioxid, heat is absorbed in the reaction. When abnormally large amounts of ammonia are eliminated in the urine, this heat

12. Grafe, E.: Zur Kenntniss des Stoffwechsels im protrahierten Hungerzustande, Ztschr. f. physiol. Chem., 1910, **65**, 48.

13. Lusk, G.: Jour. Biol. Chem., 1915, **20**, 600.

would be liberated in the body and not in the urea of the urine. Several calculations of this kind showed that such additional heat did not exceed 1 calory an hour.

The ordinary method for calculating the heat value of fat remains unchanged in spite of the abnormal production of beta-oxybutyric acid. For a liter of oxygen when used to oxidize beta-oxybutyric acid gives a heat value of 4.848 calories, if the figure, 4.69 calories per gram given by Emery and Benedict,<sup>14</sup> is adopted as the heat value of this substance. When fat itself is completely oxidized, the heat value of a liter of oxygen is 4.686, but when 100 gm. are oxidized, leaving a residue of 36 gm. of beta-oxybutyric acid, the heat value of a liter of oxygen is reduced to 4.654, a difference of 0.7 per cent.

In a former paper of this series by Allen and Du Bois<sup>1</sup> the question of the level of total metabolism in diabetes mellitus was discussed at length and need here be considered only in relation to the specific case. It has long been definitely known that an increased protein metabolism increases the heat production. Rubner<sup>15</sup> was the first to advance the idea that the increased heat production in experimental diabetes was due to an increased metabolism. Even though a large amount of the energy present in protein be eliminated in the urine as glucose, still the specific dynamic action of protein is present when it is ingested in diabetes.<sup>16</sup>

The total metabolism of Cyril K. during the period of high protein and fat ingestion was found to be 15 and 7 per cent. above the normal standard of metabolism per square meter of surface given in Paper 13 of this series. The high protein metabolism might sufficiently explain this increase.

The analysis of this factor may be accomplished in the following manner: If one assume that the usual normal excretion of protein nitrogen is 0.5 gm. per hour, one may calculate how much in excess of this the protein metabolism was during the hours of calorimeter investigation. Since Aub and Du Bois<sup>17</sup> find that in man, when 100 calories in protein are metabolized, the total heat production increases by 76.4 calories, it is possible to compute by what increment the extra metabolism of protein would increase the heat production. Such a calculation is presented in Table 8.

The first two observations on Cyril K. were made six hours after taking food. It is questionable whether the small amount ingested

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14. Emery, A. G., and Benedict, F. G.: The Heat of Combustion of Compounds of Physiological Importance, *Am. Jour. Physiol.*, 1911, **28**, 301.

15. Rubner, M.: *Die Gesetze des Energieverbrauchs bei der Ernährung*, Leipzig, 1902, p. 370.

16. Lusk, G.: *Loc. cit.*, note 6, p. 555.

17. Aub, J. C., and Du Bois, E. F.: Clinical Calorimetry, Paper 21, *THE ARCHIVES INT. MED.*, this issue.



would have much effect in stimulating metabolism at the time of observation.

It appears from this calculation that the extra heat production noted in Cyril K. was due to the extraordinarily high protein metabolism, and that with the elimination of that factor the metabolism more nearly approximated that to be expected in a somewhat emaciated individual.

The *prognosis*, as based on the laboratory findings of this case, seemed at this point to be most unfavorable and was so pronounced by Allen and by Lusk, while Joslin, who saw the patient at the same time, stated that he would recover.

*IV. Second Fasting Period.*—Following the period of high protein diet, a second fasting period was initiated, which extended over four days. The D: N ratio was 2.7, as during the first fasting period. There

TABLE 8.—SPECIFIC DYNAMIC ACTION OF EXCESSIVE PROTEIN DESTRUCTION (CYRIL K.)

Date	Urinary Nitrogen in Excess of Normal Basal per Hour	Extra Calories Due to Specific Dynamic Action (Extra $N \times 26.5 \times 0.764$ )	Total Calories per Hour	Total Calories Minus Calories of Specific Dynamic Action	Calories (minus Calories of Specific Dynamic Action) per Sq. M. per Hour	Per Cent. Average Normal
1915 12/15	0.83	16.8	81.9	65.1	36.4	— 8
12/16	0.93	18.8	76.4	57.6	32.2	—19
12/18	0.42	8.5	73.2	64.7	36.1	— 9
12/20	....	....	66.3	....	37.0	— 7
12/22	....	....	62.8	....	35.9	—10

were three distinct factors which showed improvement over the fasting period of the week before: (a) a lower protein metabolism; (b) a lower level of blood sugar; (c) a higher carbon dioxid tension of the blood. The elimination of beta-oxybutyric acid, however, did not markedly decrease until the fourth fasting day. The patient showed steady improvement throughout this period, read all day and was free from drowsiness.

*V. Convalescent Period.*—Following the period of four days of fasting, during which the D: N was 2.7, this ratio suddenly broke to 1.1, the quantity of beta-oxybutyric acid eliminated fell to 11 gm., and the respiratory quotient rose in response to the change in metabolism. Although clinically the condition of the patient had shown constant improvement, yet this day was the first to herald the overthrow of all features of the pathologic metabolism. Within a week he became permanently free from glycosuria and from acetonuria.

It is interesting to note that the excretion of beta-oxybutyric acid decreased before the fall in the D: N ratio, as appears in Table 9.

It may reasonably be questioned whether there could possibly have been an increase in the number of sugar molecules liberated in the metabolism of protein on December 21, such as to have been the cause of the improvement in the oxidation of fat, as evidenced by the fall in the quantity of beta-oxybutyric acid excreted. On the other hand, it may well be that the improvement in the conditions for the oxidation of fat may have reduced the acidosis to such an extent as to have beneficially affected the oxidation of glucose. These latter relations have been set forth in a series of papers by Murlin.<sup>18</sup>

Woodyatt<sup>19</sup> has recently stated that the decrease of acidosis in fasting, emaciated diabetics may, in part, be due to the fact that the metabolism may be solely at the expense of protein. Our observations

TABLE 9.—SHOWING FALL IN BETA-OXYBUTYRIC ACID BEFORE FALL IN D: N RATIO

Period	Date	D: N	Beta-oxybutyric Acid
IV	12/19*	2.65	56.84
	12/20*	2.44	41.17
	12/21*	2.65	26.21
V	12/22*	1.12	10.95

\* Fasting.

do not corroborate this view, for the highest amount of heat from protein yielded during periods of basal metabolism in severe diabetes has never exceeded 23 per cent. of the total heat production.

During the period of high protein-fat diet and of high acidosis the quantity of *sodium chlorid* was less in the urine than in the diet, whereas the opposite conditions obtained during the following period of fasting which resulted in the clearing up of the acidosis.

*VI. Recovery.*—After an interval of two months the patient returned to Bellevue Hospital and was again placed in the calorimeter for the determination of his basal metabolism. The day before he had partaken of a diet containing:

Protein .....	60 gm.
Carbohydrate .....	252 gm.
Fat .....	6 gm.

18. Murlin, J. R., and Kramer, B.: Pancreatic Diabetes in the Dog, Jour. Biol. Chem., 1916, **27**, 481.

19. Woodyatt, R. T.: Acidosis in Diabetes, Jour. Am. Med. Assn., 1916, **66**, 1910.

There was no sugar in the urine. The determination of the basal metabolism revealed a respiratory quotient of 0.92 and a nonprotein respiratory quotient of 0.97. These results showed that this man was now deriving his energy, 62 per cent. from carbohydrate, 31 per cent. from protein and 6 per cent. from fat. This astonishing result shows that Cyril K., who two months before had been completely diabetic, was now able to derive two-thirds of his basal requirement for energy from the oxidation of carbohydrate.

Before the onset of diabetes the weight of the patient had been approximately 73.6 kg. His minimal recorded weight as the result of the Allen fasting treatment was 45.8 kg., a loss of 27.8 kg., or 38 per cent. The normal basal metabolism of a man weighing 73.6 kg. and having a height of 188 cm. would have been 79 calories. This contrasts with an actual production of energy amounting to only 43 calories per hour when he was reduced to a weight of 45.8 kg. His heat production was therefore only 55 per cent. of what it would have been had he been of his normal weight.

The results calculated from Case 1 may be compared with those of this patient as in Table 10.

TABLE 10.—COMPARISON OF RESULTS IN CASE 1 WITH THOSE OF CYRIL K., CASE 3

Case	Height, Cm.	Original Weight, Kg.	Lowest Weight, Kg.	Lowest Heat Production in Per Cent. of the Original Normal	Variation in Per Cent. from the Normal per Sq. M. of Surface when Heat Production Was Lowest
1	169	62	32.5	40	—37
3	188	74	45.8	55	—35

After three weeks Cyril K. gained 3.5 kg. in weight, and his metabolism was found to be 27 per cent. below the normal for his weight and height, instead of 35 per cent. below when his emaciation was more extreme.

These experiments on individuals indicate how a community may long support itself on restricted rations.

#### SUMMARY

Three cases of diabetes studied in the respiration calorimeter are reported.

*Case 1.*—A diabetic woman through prolonged fasting had been brought to a level of metabolism 37 per cent. below the standard level for her height and weight, a metabolism which was only 40 per cent.



of the original normal value before the initiation of fasting. This is the lowest level of metabolism ever recorded.

*Case 2.*—A patient with a mild case of diabetes, whose urine was free from sugar, became suddenly and completely diabetic on the administration of a high protein-fat diet, and, on fasting, rapidly regained his former tolerance for sugar.

*Case 3.*—An acute case of complete diabetes which, in high protein metabolism and in high D:N ratios, resembled experimental diabetes in animals. When given meat and fat, the patient not only excreted all the sugar derivable from protein, but he also excreted all the beta-oxybutyric acid derivable from the fat metabolism of the time. Two months later when the metabolism was 35 per cent. below the normal for his height and weight, two-thirds of the total calories were derived from carbohydrate. The case has afforded an opportunity for the discussion of many of the fundamental facts concerning diabetes mellitus.

477 First Avenue.

## CLINICAL CALORIMETRY

TWENTY-FIFTH PAPER

### THE WATER ELIMINATION THROUGH SKIN AND RESPIRATORY PASSAGES IN HEALTH AND DISEASE \*

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NEW YORK

1. Introduction.
2. The Sources of Error in Technic.
3. Review of the Literature.
4. Methods Used.
5. Water Elimination of Normal Men Under Standard Conditions.
6. Water Elimination of Patients Under Similar Conditions.
7. Summary and Conclusions.

#### INTRODUCTION

Few clinicians realize the importance of water in the metabolism, and few investigators appreciate the large errors in its measurement. Apparently, there is nothing easier to measure, and this has led many to publish results without careful controls as to accuracy. As a matter of fact, the technic of determining the water of vaporization is exceedingly difficult and there are few things more laborious than the establishment of the water balance of a patient.

Rubner has pointed out that a starving animal can lose practically all of his glycogen and fat and half of his body protein. If he loses about 10 per cent. of the water of his body he dies. In the clinic few attempts are made to measure the water balance of patients whose tissues are becoming dried on account of insufficient water intake in comparison with an increased output. With edematous patients it is the custom to measure the urine and the fluids of the food with the idea of determining the gain or loss of water to the body. Such measurements are very incomplete, as will be shown by the accompanying example of a true balance sheet (Table 1).

Clinicians allow the nurses to make a large error in neglecting the water content of the so-called *solid* foods. Let us take, for example, the following meal: A small dish of farina, a boiled potato, a portion of tomato and lettuce salad, a small dish of junket and an apple. This is a supper that might well be given a nephritic patient, but it contains about 690 gm. water, something over 23 ounces.

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\* From the Russell Sage Institute of Pathology, in affiliation with the Second Medical Division, Bellevue Hospital.

TABLE 1.—ILLUSTRATING WATER BALANCE

Water Intake:	Gm.	
Drinking water .....	300	
Water in coffee, milk, soup.....	580	
Water in "solid foods" .....	720	
H <sub>2</sub> O from oxidation of 100 gm. protein.....	41	
H <sub>2</sub> O from oxidation of 110 gm. fat.....	118	
H <sub>2</sub> O from oxidation of 244 gm. carbohydrate.....	135	
		1,894
Water Output:	Gm.	
Water in urine.....	750	
Water in feces.....	300	
Water vaporized through skin and respiratory passages.....	700	
		1,750
Plus balance to body.....		144
Gain in body weight.....		100

It is the purpose of the present paper to give some idea as to the amount of water lost from the body in vaporization, and to call attention to this neglected factor in the total output. We can try to estimate the so-called *insensible* perspiration by weighing the patient every day and measuring everything taken and excreted. More accurate determinations can be made in a few cases in respiration chambers. Absolutely accurate determinations cannot be made at present, and it seems advisable to take up this point in detail before reviewing the literature of the subject.

*Alcohol Checks.*—It has been the policy of the Russell Sage Institute of Pathology to publish each year all the alcohol checks made with the calorimeter (Table 2). This enables other investigators to satisfy themselves as to the accuracy of the apparatus at any given time during the experimental season. Occasionally, there will be an error as great as 2 per cent. in some of the measurements. The average errors for the season of 1915-1916 are as follows:

	Per Cent.
Heat .....	+0.63
Oxygen .....	—0.25
Carbon dioxid .....	—0.42

Respiratory quotient 0.665 instead of the theoretical 0.6666. This shows that the calorimeter is still extraordinarily accurate. The usual error of +2.5 per cent. in measuring the water elimination will be discussed later. It is interesting to study the results in the check of April 3, when about one-quarter of the customary amount of alcohol was burned. The measurement of oxygen and carbon dioxid was surprisingly good, but the method of direct calorimetry gave an error of +2.5 calories in three hours. The large calorimeter was never intended to measure a heat production as low as 19 calories an hour, but even for such small amounts, using the method of indirect calorimetry, it compares favorably with any other respiration chamber as yet constructed.



## THE SOURCES OF ERROR IN TECHNIC

It is a very simple matter to measure the water vapor entering and leaving a respiration chamber in which a man or a part of his body is confined. The difference would represent the water vaporized from the body were it not for the fact that water clings to the walls and to the contents of every experimental cabinet. Surprisingly few investigators have taken the trouble to see just how much could be lost or gained in this manner. Stohman<sup>1</sup> called attention to the amount of water that could be absorbed by wood and painted metal. Atwater and Benedict<sup>2</sup> tested the accuracy of the water determination of the Middletown calorimeter, and later the question was discussed in detail by Benedict, Riche and Emmes<sup>3</sup> and by Benedict,<sup>4</sup> who found that large amounts of water vapor could be removed from calorimeter walls and from clothes, bedding and other materials contained within them.

In Papers 2 and 11 of this series it was shown by means of alcohol checks that the Sage calorimeter measured very accurately the oxygen, carbon dioxid and heat, but gave an average error of more than 3 per cent. in the determination of the water produced by the flame. The amount of alcohol usually burned each hour gives off about as much heat and carbon dioxid as a man and consumes about the same amount of oxygen, but produces somewhat less than half the water vaporized by most of the subjects. As a result, the air in the box becomes drier and drier and the water vapor deposited on the tinned walls of the calorimeter, on the copper cooling pipes, on the glass windows and the small wooden parts of the air thermometers, is more or less completely removed as the test progresses. During the first few hours of the experiment there may be a plus error of 0.5 to 1.0 gm. an hour, but later it seldom exceeds half a gram. When the wooden bed, pillow, folded sheet, night-shirt, socks and pajamas are in the calorimeter, astonishing amounts of water can be absorbed or given off, depending on the changes in the relative humidity of the air. In order to demonstrate this, three experiments were made. Nov. 17, 1916, the bed and clothing were placed in the calorimeter, the alcohol lamp was adjusted to burn the usual amount of alcohol per hour, and an arrangement was made by which known amounts of water could be dripped on a piece of muslin about a foot square, tacked on a small wooden frame. The total water vapor formed was estimated by adding the

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1. Stohman: *Landwirthschaftliche Versuchsstationen*, 1876, **19**, 81 and 159.

2. Atwater, W. O., and Benedict, F. G.: *A Respiration Calorimeter*, Carnegie Institution of Washington, Pub. 42, 1905.

3. Benedict, F. G., Riche, J. A., and Emmes, L. E.: *Control Tests of a Respiration Calorimeter*, *Am. Jour. Physiol.*, 1910, **26**, 1.

4. Benedict, F. G.: *A Comparison of the Direct and Indirect Determination of Oxygen Consumed by Man*, *Am. Jour. Physiol.*, 1910, **26**, 15.

TABLE 2.—ALCOHOL CHECKS—

Date	Hour	Heat				Oxygen		
		Alcohol Burned, Gm.*	Theory, Cals.	Found, Cals.	Error, per Cent.	Theory, Gm.	Found, Gm.	Error, per Cent.
10/29/15	{ 1	12.70	81.2	81.7	+0.6	23.9	24.8	+3.7
	{ 2	12.34	78.9	79.4	+0.6	23.2	22.9	-1.4
	{ 3	12.77	81.7	80.1	-2.0	24.1	23.6	+2.0
	{ 4	12.10	77.4	78.7	+1.7	22.8	23.3	+2.2
Average	...	.....	79.8	80.0	+0.2	23.5	23.8	+1.3
12/13/15	{ 1	11.17	73.5	68.8	-6.0	21.6	21.6	±0.0
	{ 2	10.44	68.7	69.1	+0.6	20.2	20.2	±0.0
	{ 3	10.38	68.3	69.7	+2.0	20.1	20.4	+1.5
Average	...	.....	70.2	69.2	-1.3	20.6	20.7	+0.5
1/19/16	{ 1	11.96	78.7	79.5	+1.0	23.2	22.9	-1.3
	{ 2	12.42	81.7	80.7	-1.2	24.1	25.3	+5.0
	{ 3	12.37	81.4	80.7	-0.8	24.0	24.2	+0.8
Average	...	.....	80.6	80.3	-0.3	23.8	24.1	+1.2
1/21/16	{ 1	11.59	76.3	78.4	+2.7	22.5	22.6	+0.4
	{ 2	11.44	75.3	76.1	+1.0	22.2	22.4	+0.9
	{ 3	11.31	74.4	77.0	+3.5	21.9	21.5	-1.8
Average	...	.....	75.3	77.2	+2.4	22.2	22.2	±0.0
2/18/16	{ 1	11.92	78.4	77.8	-0.7	23.1	22.5	-2.6
	{ 2	11.89	78.2	77.9	-0.4	23.0	23.0	±0.0
	{ 3	11.26	74.1	75.2	+1.5	21.8	21.2	-2.7
Average	...	.....	76.9	77.0	+0.1	22.6	22.2	-1.8
2/25/16	{ 1	11.82	77.8	77.5	-0.4	22.9	22.5	-1.7
	{ 2	11.47	75.5	76.1	+0.8	22.2	22.3	+0.4
	{ 3	11.73	77.2	78.0	+1.0	22.7	22.5	+0.8
Average	...	.....	76.8	77.2	+0.5	22.6	22.4	-0.9
4/ 3/16	{ 1	2.69	17.7	19.1	+7.9	5.21	4.93	-5.4
	{ 2	2.49	16.4	20.4	+24.4	4.82	5.15	+7.0
	{ 3	3.37	22.2	24.5	+10.5	6.53	6.33	-3.1
Average	...	.....	18.8	21.3	+13.3	5.52	5.48	-0.7
4/14/16	{ 1	12.63	83.1	83.3	+0.2	24.5	24.3	-0.8
	{ 2	12.35	81.3	81.1	-0.2	23.9	23.3	-2.5
	{ 3	12.26	80.7	81.3	+0.5	23.8	23.5	-1.3
Average	...	.....	81.7	81.9	+0.2	24.1	23.7	-1.6
4/17/16	{ 1	14.07	174.2	84.4	+0.3	51.3	24.8	-0.2
	{ 2	12.41	87.4	90.3	+3.3	25.7	26.4	+2.7
	{ 3	13.28	87.4	87.8	+0.5	25.7	25.4	-1.1
Average	...	.....	87.2	87.5	+0.3	25.7	25.5	-0.8
Total.....	...	.....	2,021.7	2,034.6	+0.63	565.3	593.8	-0.25

water produced by the alcohol flame to the water dripped into the box each hour. The results are shown in Table 3, and it will be seen that 24 to 28 gm. of water were lost per hour. The method of dripping water was rather crude, and much water must have been retained by the wooden frame. Even so, it was evident that the bed and clothing, which had been placed in the box when very dry, absorbed a great deal of water as the humidity rose rapidly. After this experiment was over, still more water was vaporized, the air almost

-SEASON 1915-1916

Carbon Dioxid			Water			R. Q. Theory, 0.667
Theory, Gm.	Found, Gm.	Error, per Cent.	Theory, Gm.	Found, Gm.	Error, per Cent.	
21.9	22.1	+0.9	14.7	15.4	+4.8	0.648
21.3	21.4	+0.4	14.3	14.8	+3.5	0.678
22.0	21.5	-2.2	14.8	14.8	±0.0	0.664
20.9	21.0	+0.5	14.0	14.7	+0.5	0.659
21.5	21.5	±0.0	14.1	14.9	+3.4	0.662
19.8	19.7	-0.5	12.9	12.9	±0.0	0.664
18.5	18.3	-1.0	12.1	12.4	+2.4	0.660
18.4	18.4	±0.0	12.1	12.3	+1.7	0.657
18.9	18.8	-0.5	12.4	12.5	+0.8	0.660
21.2	21.0	-0.9	13.9	14.0	+0.7	0.668
21.6	22.2	+2.7	14.4	14.6	+1.4	0.640
22.0	21.6	-1.8	14.4	14.1	-2.0	0.647
21.6	21.6	±0.0	14.2	14.2	±0.0	0.652
20.6	20.4	-0.9	13.5	13.8	+2.2	0.658
20.3	20.1	-0.9	13.3	13.5	+1.5	0.651
20.1	20.0	-0.5	13.1	13.5	+3.0	0.674
20.3	20.2	-0.5	13.3	13.6	+2.2	0.661
21.2	20.9	-1.4	13.9	13.9	±0.0	0.676
21.1	21.0	+0.4	13.6	14.1	+2.1	0.664
20.0	19.9	-0.5	13.1	13.3	+1.5	0.682
20.8	20.6	-0.9	13.6	13.8	+1.4	0.674
21.0	20.6	-1.9	13.7	14.0	+2.2	0.664
20.4	20.4	±0.0	13.3	13.7	+3.0	0.666
20.9	20.8	-0.5	13.6	13.9	+2.2	0.672
20.8	20.6	-0.9	13.5	13.9	+3.0	0.667
4.78	4.61	-3.6	2.98	3.77	+26.0	0.680
4.42	4.69	+5.6	2.89	3.63	+25.0	0.662
5.94	5.99	±0.0	3.93	4.16	+ 6.0	0.688
5.06	5.07	±0.0	3.26	3.85	+18.0	0.677
22.4	22.3	-0.4	14.7	15.0	+2.0	0.669
21.9	21.7	-0.9	14.3	14.5	+1.4	0.677
21.8	21.5	-1.3	14.2	14.5	+2.1	0.667
22.0	21.8	-0.9	14.4	14.7	+2.0	0.671
47.0	23.0	} +0.6	30.7	15.4	} +4.2	0.676
23.6	24.1			16.6		0.665
23.6	23.6	±0.0	15.4	15.8	+2.6	0.672
23.5	23.6	+0.4	15.4	15.9	+3.2	0.672
545.1	542.8	-0.42	358.0	367.1	+2.51	Av. 0.665

\* In all tests the solution contained 92.92 per cent. by weight of ethyl alcohol.

saturated and allowed to stand this way all night. The next morning the calorimeter was opened and the muslin on its wooden frame placed on a copper plate over the chimney of the alcohol lamp. The lamp was lighted, and during the preliminary period of an hour and the first experimental period of thirty minutes, water was dripped on the muslin, raising the humidity to 60 per cent., at which point there appeared the first dew on the polished pipe of the ingoing water



at a temperature of 15.9 C. The dripping of water was then discontinued, and from this time the alcohol flame was the only source of water except for the bed and clothing and wall of the box. The rate of ventilation was then increased until the relative humidity had fallen to 27 per cent. The large amounts of water removed from the calorimeter can be seen in Table 3. December 4, a complete alcohol check was made, but in addition, 20 c.c. extra water was dripped each hour on a small piece of asbestos in an enamel-ware plate over the lamp chimney. The amount of water recovered was from 1.5 to 2.6 gm. an hour less than the total vaporized. The bed and clothing, which had been exposed to comparatively dry air all night, continued to absorb some moisture for several hours after they were exposed to a greater, but uniform, humidity. In this experiment the average error in the water measurement was 6.7 per cent. It could perhaps be reduced to half of this if great care were taken to maintain an even humidity in the calorimeter room all night. This is difficult, but it is now being attempted.

TABLE 3.—MOISTURE TESTS

Length of Period, Minutes	Total H <sub>2</sub> O Formed or Dripped into Calorimeter, Gm.	H <sub>2</sub> O Removed, Gm.	Errors, Gain or Loss, Gm.	Relative Humidity at End of Period	Remarks
Nov. 17	....	....	.....	38	Water dripped into box
60	53.0	25.0	-28.0	45	
60	50.0	26.0	-24.0	44	Water dripped into box
Nov. 18	....	....	.....	54	
30	80.0	18.0	-14.0	60	Water dripped into box
30	8.0	19.0	+11.0	57	No extra water
30	9.0	18.0	+ 9.0	54	No extra water
60	16.0	40.0	+24.0	33	No extra water
60	17.0	30.0	+13.0	29	No extra water
60	17.0	27.0	+10.0	27	No extra water
Dec. 4	....	....	.....	36	
60	33.9	32.4	- 1.5	37	20 c.c. extra water dripped into box each hour
60	33.6	30.9	- 2.7	38	
60	33.2	30.6	- 2.6	36	
	100.6	93.9			

The Sage calorimeter is probably much better adapted for the accurate determination of water vaporized than any other respiration chamber except the Middletown calorimeter, which was used for periods of several days. Even so, it is doubtful if it is much more accurate than 10 per cent. under ordinary conditions.

## REVIEW OF THE LITERATURE

In reading over the many publications on the subject of water vaporization we must bear in mind the large technical errors found in all types of apparatus. Some investigators took the trouble to weigh the clothing of their subjects before and after the experiment, but could not weigh the experimental chamber and its furniture. Some made the error of drying the cabinet before an experiment, not realizing that water could be lost with a rising humidity. Some have even expressed their results in six significant figures, when it is doubtful if the second figure was accurate.

The older work on vaporization is well reviewed by Rubner,<sup>5</sup> Magnus-Levy<sup>6</sup> and Tigerstedt.<sup>7</sup> In 1866 Pettenkofer and Voit<sup>8</sup> found that the water elimination of a fasting man was 814 to 829 gm. a day. Voit's pupil, Rubner, made a very thorough study of the question, and with his assistants published a series of papers which are of great importance. Their value is increased by the fact that they were produced in the same laboratory with comparable experimental methods. Rubner<sup>9</sup> first studied guinea-pigs and dogs, using a small Pettenkofer-Voit chamber and an air calorimeter. He found that animals gave off much more water in dry than in moist air, a drop in the relative humidity from 69 to 31 per cent. increasing the water elimination from skin and lungs twofold or threefold. Water elimination in guinea-pigs increased as the temperature of the air was raised above 15 C.; in dogs it increased with temperatures above 7 C. When the dogs were given meat, thus increasing the heat production, the water elimination was not affected when the surrounding air was cool, but was markedly increased when the air was warm. Rubner found that for different animals the water elimination was not proportional to surface area, but was nearly proportional to body weight. He concludes, also, that variations in the humidity of the air do not affect the metabolism of protein and fat, and cause no significant change in the total heat production. Increasing moisture in the air diminishes the heat loss through vaporization, but correspondingly increases the heat loss through radiation and conduction.

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5. Rubner, M.: *Die Gesetze des Energieverbrauchs bei der Ernährung*, Leipzig, 1902; *Lehrbuch der Hygiene*, Leipzig, 1907.

6. Magnus-Levy, A.: In von Noorden's *Handbuch der Pathologie des Stoffwechsels*, Berlin, 1906, **1**.

7. Tigerstedt, R.: *Wärmeverlust des Körpers*, in Nagel's *Handbuch der Physiologie des Menschen*, Braunschweig, 1909, **1**, 581.

8. Pettenkofer, M., and Voit, C.: *Untersuchungen über den Stoffverbrauch des normalen Menschen*, *Ztschr. f. Biol.*, 1866, **2**, 478.

9. Rubner, M.: *Die Beziehung der atmosphärischen Feuchtigkeit zur Wasserdampfabgabe*, *Arch. f. Hyg.*, 1890, **11**, 137; *Stoffzerzetzung und Schwankungen der Luftfeuchtigkeit*, *ibid.*, p. 243; *Thermische Wirkungen der Luftfeuchtigkeit*, *ibid.*, p. 255.

TABLE 4.—WATER ELIMINATION AT 15 C.

	Weight, Gm.	H <sub>2</sub> O per Kg. per Day	Percentage of Calories Lost in Vaporization
Man.....	70,000	12.6	22.9
Dog.....	30,000	12.2	20.5
Dog.....	4,000	11.5	9.0
Guinea-pig.....	550	11.3	6.5

Cramer<sup>10</sup> found that salt was excreted by the skin even when the subjects were at rest in a room at low temperature and concluded that there was a continual secretion of insensible sweat. Nuttall<sup>11</sup> found that when a naked man was placed in a cabinet at a temperature of 29 C., changes in the humidity between 12 and 63 per cent. had little influence on the amount of water removed by the apparatus. Wolpert<sup>12</sup> found that light work scarcely increased the water elimination above the resting values, but that heavy work caused a large increase. He found that the variations in the humidity of air next to the body are limited by the clothing and are not as great as the variations in the outside air. When the air temperature was between 15 and 35 C., the wind from a fan diminished the water loss from the body; above and below these points it increased the vaporization. Laschtschenko<sup>13</sup> demonstrated that the drinking of two liters of water had no effect on the amount eliminated through skin and respiratory passages. Rubner<sup>14</sup> made some interesting determinations of the water eliminated

10. Cramer: Ueber die Beziehung der Kleidung zur Hautthätigkeit, Arch. f. Hyg., 1890, **10**, 231.

11. Nuttall: Ueber den Einfluss von Schwankungen in der relativen Feuchtigkeit der Luft auf die Wasserdampfabgabe der Haut, Arch. f. Hyg., 1895, **23**, 184.

12. Wolpert: Ueber den Einfluss der Lufttemperatur auf die im Zustand anstrengender körperlicher Arbeit ausgeschiedenen Mengen Kohlensäure und Wasserdampf beim Menschen, Arch. f. Hyg., 1896, **26**, 32; Ueber die Kohlensäure- und Wasserdampf- Ausscheidung des Menschen bei gewerblicher Arbeit und bei Ruhe, *ibid.*, p. 68; Einfluss der Luftbewegung auf die Wasserdampf- und Kohlensäure-Abgabe des Menschen, *ibid.*, 1898, **33**, 206; Ueber den Einfluss des Windes auf die Atmungsgrosse des Menschen, Arch. f. Hyg., 1902, **43**, 21; Ueber den Einfluss der Luftfeuchtigkeit auf den Arbeitenden, *ibid.*, 1899, **36**, 202. Wolpert and Peters: Die Tageskurve der Wasserdampfabgabe des Menschen, *ibid.*, 1906, **55**, 299.

13. Laschtschenko: Ueber den Einfluss des Wassertrinkens auf Wasserdampf- und CO<sub>2</sub>-Abgabe des Menschen, Arch. f. Hyg., 1898, **30**, 145.

14. Rubner, M.: Notiz über die Wasserdampfausscheidung durch die Lunge, Arch. f. Hyg., 1898, **33**, 151. Rubner, M., and Von Lewaschew: Ueber den Einfluss der Feuchtigkeitsschwankungen unbewegter Luft auf den Menschen während körperlicher Ruhe, *ibid.*, 1897, **29**, 1.



in the breath during various activities. The subject gave off 17 gm. an hour when quiet, 19 gm. when breathing deeply, 28 gm., reading, and 34 gm. singing. Schattenfroh<sup>15</sup> found that a fat man gave off much more water than a thin man in work experiments, and that with an air temperature of 38 C. and relative humidity of 63 per cent., the heat elimination was not sufficient to prevent a rise of body temperature. Rubner<sup>16</sup> makes some important calculations as to the amount of water a man in the tropics must drink in order to provide enough urine to dissolve the urea and also enough sweat to keep the man cool. It is obvious that with an air temperature of 38 C. or higher, vaporization is the only means of dissipating heat from the body. In order that heat may be dissipated, a man requires 4,400 gm. of water on the ordinary European diet, if producing 2,400 calories per day. On an exclusive meat diet he would require 7,600 gm. of water.

A large amount of data in regard to the water elimination of man can be found in the publications of Atwater, Benedict and their associates.<sup>17</sup> These investigators used a large Atwater-Rosa calorimeter in which a man lived for periods of half a day to two weeks. The experiments were not planned as a study of the water elimination, but they are of great value on account of their length and completeness.

The results are summarized by Benedict and Carpenter<sup>18</sup> who have corrected some miscalculations of the earlier publication. The temperature of the calorimeter was about 20 C., and the humidity in the rest experiments comparatively low. In the work experiments, however, the rate of ventilation was not sufficient to prevent a rise in humidity until it reached a point where there was condensation on the cold-water pipes in the ceiling. These helped to remove the moisture from the air and the water which dripped from them was collected and weighed by the subject of the experiment. The relative importance of the various channels of heat loss are calculated by Benedict and Carpenter and also the changing percentage of calories lost in vaporization with different degrees of muscular activity.

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15. Schattenfroh: *Respirationsversuche an einen fetten Versuchsperson*, Arch. f. Hyg., 1900, **38**, 93.

16. Rubner, M.: *Vergleichende Untersuchung der Hautthätigkeit des Europaers und Negers, nebst Bemerkungen zur Ernährung in hochwarmen Klimaten*, Arch. f. Hyg., 1900, **38**, 148.

17. Atwater, W. O., and Benedict, F. G.: *Experiments on the Metabolism of Matter and Energy in the Human Body*, U. S. Dept. of Agriculture, Bull. 136, 1903, pp. 95 and 134. Benedict, F. G.: *The Influence of Inanition on Metabolism*, Carnegie Institution of Washington, Pub. 77, 1907. Benedict, F. G., and Carpenter, T. M.: *The Metabolism and Energy Transformations of Healthy Man During Rest*, *ibid.*, Pub. 126, 1910. Benedict, F. G.: *A Study of Prolonged Fasting*, *ibid.*, Pub. 203, 1915.

18. See Note 17.

TABLE 5.—APPORTIONMENT OF HEAT LOSS

	Rest	Work
Radiation and conduction.....	75.8	45.5
To warm inspired air.....	2.0	
Heat of urine and feces.....	0.7	0.4
Water vaporized from lungs.....	9.8	43.6
Water vaporized from skin.....	12.2	
Heat equivalent of external muscular work.....	....	10.5

TABLE 6.—PERCENTAGE OF CALORIES LOST IN VAPORIZATION

Condition of Activity	Number of Subjects	Heat Eliminated, Cals.	Percentage of Calories Lost in Vaporization
Rest.....	5	2,266	23.8
Moderate work.....	2	3,703	30.9
Severe work.....	3	4,313	39.6
Very severe work.....	2	8,225	47.6

Benedict,<sup>19</sup> in his first work on inanition, reviews the literature on the water elimination in fasting. He found that the greatest output took place in the waking hours, and that the amount of drinking water had no effect on the amount eliminated. Benedict calculates the water elimination from the lungs by estimating the volume of the respired air and determining the amount of water required to raise its water content to saturation at body temperature. He found that in most experiments the water elimination from the lungs was somewhat less than from the skin. In the second publication on inanition<sup>19</sup> it is possible to follow the water elimination through a long period of fasting, the subject spending each night in a small bed calorimeter at a temperature of about 21 C. He calculates the water vaporized from the lungs as 9.6 to 10.7 gm. per hour, or from 40 to 65 per cent. of the total.

Day of Fast	Relative Humidity	Water Vaporized Gm.
1-3	52-63	23-29
17-20	39	15-16
27-30	42-49	18-20

19. See Note 17.

Benedict and Joslin<sup>21</sup> studied a number of diabetics in the same calorimeter and give the following figures for the percentage of calories lost in vaporization:

	Per Cent.
9 Normal controls .....	22.5
4 Severe diabetes .....	24.0
3 Light diabetes .....	22.1

In addition to the series of papers from the laboratories of Rubner and Benedict there have been a large number of scattered publications dealing with the water of vaporization. Only a few can be reviewed in this communication. Janssen,<sup>22</sup> in 1883, studied the water elimination from the arms and legs of normals and nephritics and found that vaporization was increased over edematous parts. Masje<sup>23</sup> investigated the radiation of heat from small areas of skin in different parts of the body. Erismann<sup>24</sup> placed the arm in a box and determined the influence of various factors. Von Willebrand<sup>25</sup> used a cabinet which collected the moisture from the skin only, the head of the subject projecting out of the apparatus. With a relative humidity of 40 to 50 per cent. and a temperature of 12 C., 10.5 gm. water per hour were given off by the skin; at 18 C., 13 to 18 gm.; at 28 C., 26 to 27 gm. There was a sudden outbreak of sweat in one case when the temperature reached 30 C.; in the other man, when it rose to 33.5 C. The carbon dioxid elimination from the skin was constant at 0.2 to 0.4 gm. per hour until the outbreak of sweat, when there was a marked rise in the elimination of this gas. Von Willebrand concludes that before the outbreak of visible sweat the so-called *insensible* perspiration was chiefly vaporization from the outer layers of the skin and not the secretion of the sweat glands. Schwenkenbecker<sup>26</sup> used a similar form of apparatus and confirmed the work of von Willebrand on normals. He studied patients with exophthalmic goiter, tuberculosis, nephritis, diabetes, pneumonia, ichthyosis and several other conditions. He deter-

21. Benedict, F. G., and Joslin, E. P.: *Metabolism in Diabetes Mellitus*, Carnegie Institution of Washington, Pub. 136, 1910.

22. Janssen: *Die Hautperspiration beim gesunden Menschen und bei Nephritikern*, Deutsch. Arch. f. klin. Med., 1883, **33**, 334.

23. Masje: *Untersuchungen über die Wärmestrahlung des menschlichen Körpers*, Arch. f. path. Anat., 1887, **17**, 267.

24. Erismann, E.: *Zur Physiologie der Wasserverdunstung von der Haut*, Ztschr. f. Biol., 1875, **11**, 1.

25. Von Willebrand, E. A.: *Ueber die Kohlensäure- und Wasserausscheidung durch die Haut des Menschen*, Skand. Arch. f. Physiol., 1902, **13**, 337.

26. Schwenkenbecker, Dr.: *Ueber die Ausscheidung des Wassers durch die Haut von Gesunden und Kranken*, Deutsch. Arch. f. klin. Med., 1904, **79**, 29. Schwenkenbecker and Tuteur: *Wie reagiert der fiebernde Mensch auf eine willkürliche Steigerung seiner Wärmebildung?* Arch. f. exper. Path. u. Pharmakol., 1907, **57**, 285. Schwenkenbecker and Inagaki: *Ueber die Schweisssekretion im Fieber*, *ibid.*, 1905, **53**, 365.



mined the rise in water elimination in typhoid fever after giving certain standard diets and followed the results in various stages of the disease. He concludes that with a rising temperature the vaporization from the skin is at the lower normal limit. With a continued temperature it is somewhat raised, and with a falling temperature it is "active" in proportion to the fall in temperature. In the course of a long-continued fever it becomes gradually smaller. Loewy and Wechselman<sup>27</sup> have made an important contribution in the study of three men born without sweat glands. Although they could not sweat, even when exposed to great heat, they were able at ordinary temperatures to lose as much water through the skin as normal controls. Loewy<sup>28</sup> continued the work with normal adults, children, patients with ichthyosis and two who were paralyzed. He found that different parts of the body gave off different amounts per square meter of surface, and that the same part showed large variations at different times. Anything that lowered the temperature of the skin decreased the water elimination. He believes that the insensible perspiration at ordinary temperatures is due to a physical vaporization through the outer skin and not to a secretion of the sweat glands. He confirmed the older observation of Unna that fat rubbed on the skin diminishes the water loss.

Lang<sup>29</sup> studied the influence of food and of tuberculin fever on the water elimination. He gives a good review of the Russian literature.

Stachelin,<sup>30</sup> using a Jaquet respiration apparatus, determined the heat production and water elimination in the night after various standard meals. The percentage of calories lost in vaporization was between 18 and 21 in basal and food experiments. A well planned experiment on a tuberculous patient with nightsweats was almost spoiled by the restlessness of the subject. Niemann<sup>31</sup> gives figures showing the water of vaporization of babies. This determination must present unusual technical difficulties.

Magnus-Levy<sup>32</sup> calculates the amount of water formed in the oxidation of the various foodstuffs as follows:

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27. Loewy and Wechselman: Zur Physiologie und Pathologie des Wasserwechsels und der Wärmerregulation seitens des Hautorgans, *Virchows Arch. f. path. Anat.*, 1911, **206**, 79.

28. Loewy, A.: Untersuchungen über die physikalische Hautwasserabgabe, *Biochem. Ztschr.*, 1914, **67**, 243.

29. Lang: Beobachtungen über die Wasserausscheidung durch Haut und Lungen unter den Einfluss des Fiebers, *Deutsch. Arch. f. klin. Med.*, 1904, **79**, 343.

30. Stachelin: Versuche über Gaswechsel und Energieverbrauch nach Nahrungsaufnahme, *Ztschr. f. klin. Med.*, 1906, **66**, 201; Der respiratorische Stoffwechsel eines Phthisikers während des Nachtschweisses, *ibid.*, 1906, **66**, 241.

31. Niemann: Der respiratorische Gaswechsel in Säuglingsalter, *Ergebn. der inn. Med. u. Kinderh.*, 1913, **11**, 32.

32. Magnus-Levy, A.: In von Noorden's Handbuch der Pathologie des Stoffwechsels, Berlin, 1906, **1**, 424.

	Gm.	For 100 Calories
100 gm. fat give .....	107.1	11.3
100 gm. starch give .....	55.5	13.3
100 gm. protein give .....	41.3	9.3
100 gm. alcohol give .....	117.4	16.8

On the ordinary mixed diet of 2,500 calories we should, therefore, obtain about 300 gm. water from the oxidation of the hydrogen in the foods.

The extensive literature on the vaporization of water in fever is not reviewed, but will be discussed in a later communication.

#### METHODS USED

The Sage calorimeter was built primarily to determine the oxygen consumption, carbon dioxid elimination and total heat production. This latter, as determined by the method of direct calorimetry, is the algebraic sum of the heat of radiation and conduction, the heat stored in or lost from the body as the temperature rises or falls, and the heat rendered latent in the vaporization of water. The air within the calorimeter in all the experiments is between 22 and 24 C., and at this temperature the vaporization of 1 gm. of water requires 0.584 calories. If 1 gm. of water is condensed, the same amount of heat is liberated. For this reason the calorimeter measures accurately the total heat eliminated from the subject, even though some of the water vaporized by the skin be deposited on the walls, pipes or bedding. In such a case, part of the calories which are actually lost from the body by the vaporization of water are transferred to the column of "radiation and conduction." Fortunately, this error is not very large until the humidity reaches a point near 60 per cent., when there is condensation of visible dew on the pipes in the ceiling. The water enters these at a temperature of 13 to 16 C. and leaves them 2 to 4 degrees higher, removing in this manner the heat of radiation and conduction. Their surface area is great and they condense large amounts of moisture from the air when it is saturated at their temperature.

Sometimes the patient is sealed in the calorimeter when the walls and bedding have stood in air at a certain degree of humidity, and then the air is made drier by excessive ventilation. This removes not only the water vaporized from the body, but also a certain amount of moisture from the bedding, etc. More frequently, the humidity rises and moisture is absorbed by the contents of the calorimeter. The subject has been discussed under the heading of "Sources of Error in Technic."

We find ourselves, then, with about 300 experiments on patients and normal controls in which the water vapor removed in the ventilating current of air had been determined in hourly periods. No other

group of observations furnishes so much information as to the water vaporized from skin and respiratory passages in disease. The interpretation of the results must be made with great care on account of the unavoidable technical error.

At the very beginning we must put aside certain experiments and exclude them from the tables used in obtaining averages. The first to be ruled out are those in which the water elimination was so great that there was a condensation of moisture on the cold-water pipes. In this group we find a number of experiments on hyperthyroidism, dyspneic conditions and typhoid fever with falling temperature. These are just the cases in which we should like most to measure the increased percentage of calories lost in vaporization. We must also exclude a few cases with very low water output, because the humidity diminished so rapidly. It seems best to retain only the observations in which the relative humidity did not change more than 10 per cent. and did not approach the point of condensation on the cold pipes. This leaves us 175 satisfactory experiments. Using these, we can compare the normal controls and various groups of patients, because they were studied under certain standard conditions which tend to make the errors constant, thus allowing us to compare the results. These conditions are as follows:

The open calorimeter containing the bed, described in Paper 11, is kept at a temperature of 23 to 24 C. on the night before each experiment, the relative humidity of the air during most of the year being rather low, 15 to 40 per cent. At about half past 9 in the morning the subject is dressed in nightshirt, thick cotton pajamas and two pairs of socks; one pair cotton, one woolen. At about half past 10 he is placed in the calorimeter, the apparatus sealed, and the ventilation adjusted at a rate suitable to the estimated heat production. About an hour later the experiment is started. During this preliminary period the humidity usually rises 10 to 20 per cent., and, after the experiment has begun, continues to increase very slowly.

Before October, 1916, the Crowell blower used on the absorber table could not give a rate of ventilation higher than 70 liters per minute, and this was not sufficient to prevent a slight increase in humidity as the experiment progressed. If the subject had a very high metabolism and water elimination, the humidity would increase to 60 to 70 per cent., at which point there would be a condensation on the pipe which contained the ingoing water at a temperature of 13 to 16 centigrade.

In the summer of 1916 a Lehman Brothers' blower was installed. With this it is possible to obtain any rate of ventilation between 50 and 90 liters a minute. Higher speed would be impracticable with the present system of absorbing bottles. Using this new blower, it is possible to remove 50 to 60 gm. of water an hour and prevent condensation. It is also possible to maintain a low and absolutely uniform



humidity with a normal subject, regulating the ventilation according to the reading of a small hair hygrometer suspended just inside the window of the calorimeter. An experiment performed on E. F. D. B., Dec. 17, 1916, using the newer experimental conditions, in which the humidity was maintained very constant, showed that he gave off 37 gm. of water an hour, losing in this manner 29 per cent. of his calories. With the old blower and lower rate of ventilation and increasing humidity, an average of 31 gm. was removed per hour, and the apparent percentage loss in vaporization was 25 per cent.

An effort has been made to keep the tables expressing the results as small as possible. Only those experiments are used which have already been described in detail, and the first three columns enable one to find the paper of this series in which the data have been published. The relative humidity at the start and finish of each experiment was determined by passing 10 liters of air through a U-tube containing concentrated sulphuric acid. The actual weight of water removed from the calorimeter was determined by weighing the large Williams' bottles half filled with sulphuric acid. Finally, the percentage of calories lost in vaporization was calculated, using the method of direct calorimetry to obtain the total calories. Since the latent heat of vaporization enters into the direct calorimetry, it seemed advisable to use this method of total heat production instead of the method of indirect calorimetry which, after all, gives almost identical results in every group of experiments. Comparisons between various groups are made in the terms of percentage of heat lost in vaporization. This had been the standard method of Atwater and Benedict, and it is the logical method, since the chief function of the vaporization of water is the removal of a certain proportion of the heat produced within the body. We might express the findings in terms of grams of water per kilogram, or square meter of surface area, but this would be of no particular significance in those conditions in which the total metabolism is increased or decreased.

#### WATER ELIMINATION OF NORMAL MEN UNDER STANDARD CONDITIONS

Thirteen normal men were studied in twenty-nine experiments under the standard conditions (Table 7). They gave off an average of 29 gm. of water an hour, and lost in this manner an average of 24 per cent. of their calories. The percentage figures all come between 21 and 28, and these extremes may be considered as representing roughly the normal limits for the given conditions. It is interesting to note that the only woman studied gave off much more water. This may have been the result of different clothing.

Some of the normal men were given meals containing various

TABLE 7.—NORMAL CONTROLS

Name	Date	Details Pub- lished in Paper	Relative Humidity		H <sub>2</sub> O, Gm. per Hour	Per Cent. Calories Lost in Vapor- ization	Remarks
			Start	End			
E. F. D. B. ....	3/13/13	4	50	54	27	21	Basal experiments
	5/17/13	4	52	45	32	26	
	3/30/14	4	37	39	31	23	
	5/18/14	4	35	37	28	24	
	5/ 6/15	11	38	35	32	26	
	5/ 7/15	11	44	36	33	26	
	4/12/16	20	43	44	32	23	
	4/26/16	20	41	40	30	27	
John L. ....	3/26/14	11	36	30	25	24	
	4/ 3/14	11	28	30	24	23	
Charles M. ....	5/ 4/14	4	36	34	28	23	
Albert G. ....	12/21/14	11	34	36	32	26	
	12/23/14	11	25	32	26	24	
	12/28/14	11	29	30	26	21	
	1/ 8/15	11	32	37	29	25	
	1/ 9/15	11	38	38	33	28	
Richard S. ....	4/19/15	11	36	32	26	21	
	4/21/15	11	32	30	25	20	
Morris S. ....	12/17/14	13	27	33	27	23	
A. F. O. ....	1/21/15	13	31	33	27	26	
J. H. M. ....	4/21/16	20	40	41	34	25	
J. C. F. ....	4/27/16	20	41	34	28	22	
	11/10/16	Unpub.	35	27	25	21	
	11/13/16	Unpub.	38	33	29	24	
J. C. A. ....	4/23/16	20	35	36	30	23	
S. K. ....	5/16/16	21	39	32	27	28	
G. F. B. ....	5/23/16	21	42	37	29	26	
Average.....	.....	..	..	..	29	24	
Emma W. ....	5/13/15	13	43	35	30	34	Normal woman
	5/17/15	13	38	34	29	33	

amounts of dextrose or protein, thus increasing the heat production (Table 8). In a few cases the water elimination rose to such an extent that the ventilating current could not prevent a condensation on the pipes. In the majority of cases the table shows that the increase in water was merely proportional to the rise in total heat production. It will be noted that large doses of caffeine caused a slight rise in the water of vaporization (Table 9).

TABLE 8.—NORMAL MEN. HEAT PRODUCTION INCREASED BY GIVING DEXTROSE OR PROTEIN

Name	Date	Details Published in Paper	Relative Humidity		H <sub>2</sub> O, Gm. per Hour	Per Cent. Calories Lost in Vaporization	Remarks
			Start	End			
E. F. D. B. ....	4/ 1/14	4	37	41	32	23	After 10.5 gm. N.
	5/ 8/14	4	43	41	33	25	After 200 gm. dextrose
	5/15/14	4	36	38	30	23	After 200 gm. dextrose
Charles M. ....	5/ 6/14	4	45	41	33	26	After 200 gm. dextrose
Albert G. ....	1/ 4/15	13	29	35	31	21	
	1/ 6/15	13	35	38	33	26	
Morris S. ....	12/18/14	13	36	34	32	25	After 9.66 gm. N.
S. K. ....	5/12/16	21	29	35	25	23	After 22 gm. N.
Average.....	.....	..	..	..	32	23	

TABLE 9.—NORMAL MEN AFTER CAFFEIN

Name	Date	Details Published in Paper	Relative Humidity		H <sub>2</sub> O, Gm. per Hour	Per Cent. Calories Lost in Vaporization	Remarks
			Start	End			
E. F. D. B. ....	4/12/16	20	44	50	37	26	Caffein
	4/25/16	20	40	42	34	25	Caffein
J. H. M. ....	4/21/16	20	41	53	40	25	Caffein
J. C. F. ....	4/27/16	20	34	38	38	28	Caffein
J. C. A. ....	4/29/16	20	36	39	35	28	Caffein
Average. ....	.....	..	..	..	36	26	

TABLE 10.—BOYS AGED 12 AND 13

Name	Date	Details Published in Paper	Relative Humidity		H <sub>2</sub> O, Gm. per Hour	Per Cent. Calories Lost in Vaporization	Remarks
			Start	End			
Fabian R. S. ....	3/20/15	12	33	33	29	28	Basal
J. D. D. B. ....	3/26/15	12	33	32	26	26	Basal
Raymond M. ....	4/ 3/15	12	32	34	26	27	Basal
Reginald F. ....	4/ 5/15	12	37	36	28	27	Basal
Henry B. ....	4/ 6/15	12	39	38	33	32	Basal
Harry K. ....	4/ 7/15	12	32	37	27	27	Basal
Arthur A. ....	4/ 8/15	12	26	24	22	22	Basal
Leslie B. ....	4/ 9/15	12	30	28	23	27	Basal
Average.....	.....	..	..	..	27	27	



With the boys 12 and 13 years old it was possible to maintain a very even humidity. Their water elimination was close to the upper limit of the normal adults (Table 10). The group of very old men gave almost exactly the same figure (Table 11). The rate of ventilation was better suited to the low heat production of the boys and old men than to the greater caloric output of the men in the prime of life. A proportional ventilation might have brought the figures even closer together. Two of the dwarfs, Raphael de P. and Pat W., showed no thyroid involvement. These, grouped with the two legless men, gave the same average as the men of normal shape and size (Table 12). The other three dwarfs, with symptoms of hypothyroidism, showed a slight diminution of water elimination.

TABLE 11.—VERY OLD MEN

Name	Date	Details Published in Paper	Relative Humidity		H <sub>2</sub> O, Gm. per Hour	Per Cent. Calories Lost in Vaporization	Remarks
			Start	End			
Andrew O'C. ....	5/ 8/16	19	44	40	34	28	Old man
Henry L. ....	3/13/16	19	39	48	33	26	Old man
Charles H. ....	5/ 3/16	19	38	34	27	28	Old man
Charles W. ....	5/ 4/16	19	40	34	26	29	Old man
William C. ....	3/ 2/16	19	27	32	24	23	Old man
John B. ....	11/ 4/15	19	24	27	22	26	Old man
Average.....	.....	..	..	..	28	27	

TABLE 12.—DWARFS AND LEGLESS MEN

Name	Date	Details Published in Paper	Relative Humidity		H <sub>2</sub> O, Gm. per Hour	Per Cent. Calories Lost in Vaporization	Remarks
			Start	End			
Robert L. ....	12/ 9/14	21	30	29	24	24	Legless man
Harry J. ....	12/11/14	21	29	27	25	24	Legless man
Harry J. ....	5/ 5/16	21	35	32	25	28	Legless man
Raphael DeP. ...	3/15/16	21	30	33	22	25	Achondroplastic dwarf
Patrick W. ....	3/24/16	21	22	29	17	20	Rachitic dwarf
George F. ....	1/ 7/16	21	20	20	16	20	Pituitary dwarf;
Erwin E. ....	3/ 6/16	21	16	17	13	21	cretin (?)
Benjamin L. ....	4/10/14	14	21	20	11	21	Cretin
	4/28/14	14	19	18	11	24	Cretin

TABLE 13.—TYPHOID FEVER PATIENTS

Name	Date	Details Published in Paper	Relative Humidity		H <sub>2</sub> O, Gm. per Hour	Per Cent. Calories Lost in Vaporization	Rectal Temp. at Start, C.	Change During Exper., C.	Typhoid Period
			Start	End					
Morris S.	10/29/13	7	49	49	34	24	39.6	−0.1	Contin. temp.
	11/17/13	7	35	38	25	21	38.5	+0.5	1st relapse, ascending temp.
	11/18/13	7	38	43	28	22	39.7	+0.4	1st relapse, ascending temp.
	12/13/13	7	27	31	19	18	37.1	0.0	8th day, normal temp.
	12/16/13	7	27	32	21	20	37.3	0.0	11th day, normal temp.
	12/20/13	7	33	40	26	16	39.3	+0.5	2d relapse, early steep curve
	12/23/13	7	36	37	26	19	38.0	+0.6	2d relapse, early steep curve
	1/ 2/14	7	26	31	21	26	37.0	+0.1	8th day, normal temp.
	1/27/14	7	31	33	22	19	37.1	−0.1	38d day, normal temp.
Charles F.	11/15/13	7	37	47	31	23	40.0	+0.1	Contin. temp.
	11/29/13	7	43	40	28	24	36.7	+0.5	Early steep curve
	12/26/13	7	38	37	26	20	36.8	0.0	25th day, normal temp.
	12/31/13	7	30	37	25	22	37.1	−0.1	30th day, normal temp.
Howard F.	11/ 7/13	7	31	34	24	23	39.7	−0.2	Ascend. temp.
	11/13/13	7	33	35	23	22	39.8	0.0	Contin. temp.
	11/20/13	7	42	37	27	28	39.3	−0.4	Contin. temp.
	12/ 2/13	7	28	27	18	22	36.8	+0.1	6th day, normal temp.
	12/ 6/13	7	26	27	18	21	37.0	0.0	10th day, normal temp.
	12/18/13	7	26	35	21	21	37.1	+0.1	22d day, normal temp.
	12/30/13	7	31	35	23	23	37.3	−0.1	34th day, normal temp.
Thomas B.	10/15/13	7	34	42	26	20	36.8	+1.0	Late steep curve
	10/21/13	7	37	42	25	28	36.7	−0.1	1st day, normal temp.
Anton K.	10/16/13	7	52	49	32	27	37.0	+0.2	Late steep curve
Edward B.	10/23/14	7	41	38	30	23	38.1	+0.8	1st relapse, early steep curve
	10/27/14	7	36	35	30	26	37.4	+0.5	1st relapse, late steep curve
	11/ 4/14	7	40	37	32	27	37.2	+0.2	4th day, normal temp.
	11/ 6/14	7	38	35	25	23	38.8	+0.7	2d relapse, ascend. temp.
John K.	12/15/14	7	36	42	37	24	39.0	+0.2	Contin. temp.

#### WATER ELIMINATION OF PATIENTS UNDER STANDARD CONDITIONS

In typhoid fever the heat production is increased 30 to 50 per cent., and this makes it difficult to remove all the moisture from the calorimeter, even if the percentage of heat lost in vaporization be not raised. When the patient's temperature begins to fall there is such an increase in water eliminated from the skin that the humidity of the air rises rapidly, often to the point of condensation on the cooling pipes. For

this reason many typhoid experiments are excluded, leaving only those in which the water output was relatively low. If we group those experiments in Table 13 in which the temperature rose 0.3 to 1.0 C., we find the water elimination was decreased to the lower normal limits, 22 per cent. With a temperature that is stationary or changing only 0.2 C. in three hours, the vaporization is about the same as that of normal men, 24 per cent. With a falling temperature there is an increase which may be very marked. After the first week in convalescence the average figure is low, 21 per cent. The findings of Schwenkenbecker<sup>33</sup> are confirmed.

TABLE 14.—PATIENTS WITH EXOPHTHALMIC GOITER

Name	Date	Details Pub- lished in Paper	Relative Humidity		H <sub>2</sub> O, Gm. per Hour	Per Cent. Calories Lost in Vapor- ization	Remarks
			Start	End			
Max W. ....	3/23/14	14	39	48	36	22	Severe hyperthyroid- ism
	4/ 6/14	14	38	54	35	21	
	4/24/14	14	46	55	37	20	
Edwin T. ....	3/ 6/15	14	49	42	35	25	Acute, moderately severe
	3/10/15	14	39	40	31	25	
	3/22/15	14	47	51	38	26	
James McE. ....	3/12/15	14	53	54	43	27	Severe
	3/31/15	14	32	39	32	21	
G. S. L. ....	4/ 9/14	14	37	43	30	17	Severe
	4/16/14	14	46	53	27	21	
Peter N. ....	4/14/15	14	35	35	28	22	Atypical
Anna R. ....	5/10/13	14	44	54	32	30	Moderately severe
	5/14/14	14	40	38	30	25	
Sarah M. ....	4/29/14	14	52	50	38	30	Mild
Marion B. ....	2/ 5/15	14	40	47	36	26	Mild
Margaret L. ....	4/20/14	14	55	54	41	29	Atypical
Bessie H. ....	4/ 8/14	14	33	35	27	26	Atypical
	4/13/14	14	25	30	21	22	Atypical
	4/18/14	14	32	32	28	26	Atypical

Very recently some experiments have been made on erysipelas patients, using the improved system of ventilation made possible by the new Lehman blower. In one case the body temperature fell 1.2 C. in eight hours, and the percentage of calories lost in vaporization was 27.

33. Schwenkenbecker and Inagaki: Ueber die Schweissekretion im Fieber, Arch. f. exper. Path. u. Pharmacol., 1905, **53**, 365.



In another case the temperature rose 0.8 C. in six hours and 24 per cent. of the calories were used for vaporization.

*Exophthalmic Goiter.*—In Paper 14 of this series a table was given showing the water elimination of the patients, and the results were briefly discussed. Reviewing the findings critically (Table 14), it is necessary to rule out a large number of experiments in which the water elimination was so great that the ventilating system could not remove the moisture quantitatively. In some of these there may have been no increase in the percentage of heat lost in vaporization. In others there was undoubtedly a rise in water elimination out of proportion to the increase in radiation and conduction. These are the cases which interest us most and it is unfortunate that we cannot determine the water output. Still there remain ten experiments on four men with severe or moderately severe hyperthyroidism in which the technic was satisfactory. Of these, Dr. G. S. L. showed a diminished water elimination, losing only 19 per cent. of his calories in this manner. The other three, Max W., Edwin T., and James McE., were within normal limits in these experiments. We must remember that it was on account of high water elimination that it was necessary to exclude five out of eight observations on Max W., two out of five on Edwin T., and one out of three on James McE. We may conclude that the water elimination in severe hyperthyroidism is usually within normal limits, occasionally decreased, but very often distinctly increased.

TABLE 15.—PERNICIOUS ANEMIA

Name	Date	Details Pub- lished in Paper	Relative Humidity		H <sub>2</sub> O, Gm. per Hour	Per Cent. Calories Lost in Vapori- zation	Remarks
			Start	End			
Daniel V. ....	5/ 9/13	15	49	48	32	27	Hb 25 per cent.
Andrew K. ....	3/11/14	15	24	32	24	19	Hb 20 per cent.
	3/27/14	15	38	36	29	24	Hb 20 per cent.
Martin O. ....	5/ 4/15	15	34	32	27	27	Hb 21 per cent.
Bartolo D. ....	3/15/15	15	19	20	17	19	Hb 44 per cent.
Daniel O'O. ....	4/26/15	15	39	38	32	30	Hb 40 per cent.

*Pernicious Anemia.*—Table 15 gives the averages obtained in a series of experiments on patients with pernicious anemia. The average figure, 25 per cent., is almost identical with the standard for normal controls. This shows that the dilution of the blood that is found in severe anemia has no effect on the amount of water vaporized.

TABLE 16.—CARDIONEPHRITIC PATIENTS \*

Name	Date	Details Published in Paper	Relative Humidity		H <sub>2</sub> O, Gm. per Hour	Per Cent. Calories Lost in Vaporization	Remarks
			Start	End			
Armon W. ....	2/13/15	16	31	34	28	23	Mitral disease; fibrillation
Fred D. ....	2/15/15	16	44	45	37	29	Mitral disease; decompensated
	2/23/15	16	34	31	25	26	Mitral disease; compensated
Edward M. ....	2/17/15	16	44	42	37	25	Mitral disease; fibrillation
Charles L. ....	2/19/15	16	38	48	37	24	Valvular disease; nephritis
Burel P. ....	3/24/15	16	40	30	32	23	Valvular disease
August F. ....	5/ 1/15	16	34	31	26	31	Nephritis; fibrillation
William S. ....	2/10/15	16	25	29	24	21	Adherent pericardium
George M. ....	4/ 7/13	16	45	46	24	24	Chronic nephritis
	3/24/14	16	30	32	24	25	
	4/ 4/14	16	27	29	23	23	
David K. ....	3/21/13	16	55	52	36	24	Chronic nephritis
	3/24/13	16	49	50	28	23	
William A. ....	1/25/15	16	34	35	33	28	Aortic insufficiency
	1/27/15	16	44	36	32	27	
Theodore S. ....	1/28/14	16	56	54	39	29	Mitral stenosis; fibrillation
	1/30/14	16	52	56	37	29	
	2/ 9/14	16	31	35	22	24	

*Cardionephritic and Nephritic Patients.*—The results obtained in a series of patients in which the cardiac element predominated over the nephritic are given in Table 16. It will be seen that the water elimination was increased above the normal in almost all who suffered from moderately severe dyspnea, and in several of those with slight or no dyspnea. We should expect the increased rate of ventilation of the lungs to remove more water by vaporization, but it is quite possible that the skin is also more active. The average for the whole group was 26 per cent.

The results in fourteen experiments on nephritic patients are grouped in Table 17. The average figure, 23 per cent., is only slightly below the standard. It is instructive to group the findings according to the leading symptoms (Table 18).

Since the dyspnea must increase the vaporization from the lungs, we are forced to believe that there is a slight diminution in the water elimination from the skin in severe nephritis. Still we must remember that the total eliminated in vaporization is the same as in the case of a normal man. The waterlogged patient whose kidneys excrete perhaps

TABLE 17.—FOURTEEN NEPHRITIC PATIENTS

Name	Date	Details Published in Paper	Relative Humidity		H <sub>2</sub> O, Gm. per Hour	Per Cent. Calories Lost in Vaporization	Remarks
			Start	End			
Joseph Un. ....	3/29/16	22	40	43	35	25	Chronic parench. nephritis
	4/ 5/16	22	43	38	28	23	
	4/10/16	22	29	29	22	16	
Lee H. ....	12/ 4/15	22	27	31	24	27	Chronic parench. nephritis
	1/ 5/16	22	25	30	21	23	
Edna S. ....	12/ 1/15	22	22	22	18	26	Chronic parench. nephritis
Adam D. ....	1/14/16	22	26	30	22	22	Chronic parench. nephritis
	2/ 7/16	22	28	30	22	25	
William S. ....	12/ 6/15	22	30	34	27	21	Chronic parench. nephritis
John K. ....	3/ 4/16	22	30	33	24	22	Chronic interstitial nephritis
Isadore R. ....	1/24/16	22	38	42	18	25	Chronic interstitial nephritis; uremia
Frank C. ....	2/28/16	22	25	34	24	23	Chronic interstitial nephritis; uremia
John C. ....	2/ 9/16	22	37	37	32	25	Chronic interstitial nephritis
Mildred C. ....	12/10/15	22	32	34	27	24	Chronic interstitial nephritis

TABLE 18.—FINDINGS IN NEPHRITIC PATIENTS GROUPED ACCORDING TO LEADING SYMPTOMS

Patients with	Percentage of Calories Lost in Vaporization		
	Cardionephritis Group	Nephritic Group	Both Groups
Marked edema.....	28	24	25
Slight edema.....	25	22	23
No edema.....	26	24	24
Marked dyspnea.....	28	24	26
Slight dyspnea.....	23	23	23
No dyspnea.....	26	23	25
Whole group.....	26	23	24

100 to 400 c.c. a day, eliminates 700 to 800 c.c. water through skin and lungs. It would be interesting to know exactly the effects of hot packs and hot air baths in this disease. Theoretically, we should expect the greatest water vaporization at the highest temperature and the lowest humidity. With increasing humidity the loss from the lungs should decrease and the visible sweat should increase. Rubner and



his associates repeatedly emphasize the depressing effects of moist warm air and the comparative stimulation of dry air.

*Diabetes.*—In nine out of a total of twenty-nine basal experiments in severe and mild diabetes the percentage of calories lost in vaporization was within normal limits. In ten, the figures were above normal; in nine, below. We must remember that the extremely low metabolism

TABLE 19.—DIABETES

Name	Date	Details Published in Paper	Relative Humidity		H <sub>2</sub> O, Gm. per Hour	Per Cent. Calories Lost in Vaporization	Remarks
			Start	End			
Gerald S. ....	11/ 9/14	17	24	25	21	24	Severe, D:N 1.53
	11/12/14	17	26	28	24	27	Severe, second fast day
	11/14/14	17	20	22	18	23	Severe, fourth fast day; body dried
	11/16/14	17	39	30	28	35	Severe, sixth fast day; body dried
	11/18/14	17	15	17	14	25	Severe, eighth fast day; body dried
	11/23/14	17	15	17	13	21	5th day of feeding; beginning edema
	11/24/14	17	15	17	14	22	6th day of feeding; slight edema
	11/25/14	17	18	18	15	25	7th day of feeding; slight edema
	11/30/14	17	28	25	21	29	Convalescent
	12/ 2/14	17	30	27	24	36	Convalescent
	12/ 4/14	17	22	22	19	24	Convalescent
	12/ 7/14	17	22	22	19	24	Convalescent
William G. ....	1/11/15	17	21	20	16	22	Sugar-free; emaciated
	1/15/15	17	32	31	24	31	D:N 3.8; emaciated
	1/22/15	17	19	21	18	21	D:N 3.12; emaciated
Joseph U. ....	2/ 1/15	17	24	21	18	29	Sugar-free; emaciated
Edward M. ....	10/30/14	17	31	32	26	27	Well nourished
Felix K. ....	2/11/14	17	33	32	22	20	Sugar-free
	2/26/14	17	25	31	20	16	Sugar-free
	3/20/14	17	22	27	20	19	Sugar-free
Cyril K. ....	12/16/15	24	34	41	32	23	Severe diabetes; acidosis
	12/18/15	24	43	35	30	23	Severe diabetes; acidosis
	12/20/15	24	24	25	21	18	Severe diabetes; acidosis
	12/22/15	24	27	27	21	19	Severe diabetes; acidosis
	2/16/16	24	17	20	15	17	Recovering
	3/ 8/16	24	27	28	21	29	Recovering
Joseph D. ....	11/15/15	24	34	35	28	31	Severe diabetes
	11/17/15	24	29	35	24	20	Severe diabetes
	11/19/15	24	46	45	34	36	Severe diabetes
Anna H. ....	3/31/16	24	27	24	16	32	Much emaciated
Average.....	.....	..	..	..	21	25	

of most of the starved diabetics was accompanied by a very low water output. This in turn made the humidity of the air in the calorimeter much lower than usual, and normal controls, with the relative humidity of 15 to 25 per cent. found in some of the diabetic experiments, might have shown a water elimination somewhat above the standard of 24.1 per cent. obtained when the humidity was 35 to 50 per cent. For this reason the comparison of the starved diabetics with the normals is unsatisfactory. The results in all the experiments are given in Table 19 and the averages for the different phases of the disease are given in Table 20, omitting experiments in which the body temperature changed more than 0.2 C.

TABLE 20.—AVERAGE VAPORIZATION IN DIFFERENT PHASES OF DIABETES

	Number of Observations	Average Percentage of Calories in Vaporization
Severe Diabetes:		
After recent food.....	1	25
Basal determination.....	3	24
Second to eighth day of fast.....	6	25
Sugar-free		
Fasting.....	1	36
Basal (emaciated).....	8	26
Mild diabetes:		
Well nourished.....	3	18

The averages in severe diabetes are almost exactly the same as the normal standard, but the variations are large. The amount of water eliminated from the lungs would depend on the rate of ventilation and would be increased in patients with hyperpnea.

#### SUMMARY AND CONCLUSIONS

The technic of determining the water eliminated from the skin and respiratory passages of a subject in a respiration chamber is exceedingly difficult. If the humidity of the air in the box rises, moisture will be deposited on the contents of the chamber. If the relative humidity falls, water will be removed which was not eliminated by the subject in the experimental period.

Over three hundred experiments have been made in the Sage calorimeter on patients and normal controls at an air temperature of 22 to 25 C. and a relative humidity usually between 30 and 50 per cent. For purposes of comparison all experiments are excluded in which the relative humidity changed more than 10 per cent. between the start and finish of the observation.

TABLE 21.—WATER VAPORIZATION OF DIFFERENT GROUPS OF SUBJECTS STUDIED UNDER STANDARD CONDITIONS OF CLOTHING, TEMPERATURE AND VENTILATION. SUMMARY TABLE

Group of Subjects	Number of Experiments in Group	Percentage of Calories Lost in Vaporization
13 normal men, ages 22 to 44 years.....	29	24
6 men after glucose or protein.....	9	23
4 men after 7 to 10 grains of caffeine.....	5	26
8 boys, ages 12 to 13 years.....	8	27
6 old men, ages 77 to 88 years.....	6	27
2 legless men .....	2	25
2 dwarfs (1 rachitic; 1 achondroplastic).....	2	23
3 cretinoid dwarfs .....	4	22
4 typhoid patients, temperature rising 0.3 to 1.0 C. ..	9	22
5 typhoid patients, temperature change less than 0.3 C. during experiment .....	6	24
1 typhoid patient, temperature falling 0.4 C. ....	1	28
3 typhoid patients, 2d to 5th week of convalescence..	9	21
5 pernicious anemia patients .....	6	25
17 cardionephritics and nephritics.....	24	24
With marked edema.....	8	25
With slight edema.....	5	23
With no edema.....	8	24
With marked dyspnea.....	6	26
With slight dyspnea.....	10	23
With no dyspnea.....	8	25
8 diabetics .....	30	25

Normal men 20 to 50 years old under the standard conditions excrete on an average 29 gm. water an hour (about 700 gm. a day) through skin and air passages, losing in this manner 24 per cent. of the total heat produced. Few normal men depart more than one-tenth from this figure. All the results on groups of patients are compared with this standard figure of 24 per cent., plus or minus one-tenth. (Table 21.) Boys 12 to 13 years old give figures close to the upper limit and very old men lose almost the same percentage of calories in vaporization. Dwarfs and legless men are also close to the normal average. Cretinoid dwarfs show diminished water elimination. Typhoid patients with a rising temperature also have a decreased water output; those with a falling temperature lose an increased percentage of calories in vaporization. The water output in convalescence is low.

Some patients with hyperthyroidism have a decreased water out-



put; most of them lose the normal percentage of calories in vaporization. Some lose much more than normals in this manner. In pernicious anemia the water of vaporization is not affected. Cardiac and nephritic patients on the whole give figures close to the normal. There is a slight increase in dyspneic patients. Edema seems to have no effect on the water output through the skin. The results in diabetes show great variations. The average figure, however, is about the same as that obtained in normals.

In conclusion it may be said that the output of water is very little affected in disease. When the heat production is increased the body responds and dissipates the usual percentage of calories in the vaporization of water. When it is necessary to get rid of unusual amounts of heat the percentage lost in vaporization is increased.

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## HAY-FEVER AND HAY-FEVER POLLENS \*

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NEW ORLEANS

The causative relationship of pollens to hay-fever has become so well established that it appears almost superfluous to offer additional evidence. The experience of the past year in the correspondence of the American Hay-Fever-Prevention Association, however, has shown that there are still many who doubt that hay-fever is caused by pollen, and that there are many more who believe that pollen is only one of the exciting causes of this disease. Under the circumstances, a brief review of the most important proofs of this relationship will be of advantage.

In hay-fever, as in many other pathologic conditions, we have to consider, in addition to the exciting cause, the predisposing factors. In the greater portion of the United States there are at certain seasons many pollens in the atmosphere, which are inhaled by all in the course of normal respiration. Of this number, only a certain proportion of persons (about 1 per cent. in most states) develop hay-fever, the remainder apparently not being inconvenienced by these pollens. Those who develop hay-fever evidently have some predisposition or lower resistance, which causes them to develop hay-fever while others are not affected. That this predisposing factor is still unsettled is evidenced by the fact that in the questionnaire recently sent out by the United States Public Health Service<sup>1</sup> in Louisiana, fifty-four physicians gave forty-eight different predisposing causes for hay-fever.

### EXPERIMENTAL HAY-FEVER

The reaction of hay-fever, in no way differing from the normal attack at the usual season, except that the length and degree of the reaction is under control, may be artificially produced at any time. In the biologic laboratory of the American Hay-Fever-Prevention Association, pollens of all the most important hay-fever plants are kept in stock, so that the effect of the various hay-fever pollens may be accurately compared.

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1. Scheppegrell, W.: Hay-Fever in Louisiana, New Orleans Med. and Surg. Jour., October, 1916.



In producing an artificial attack of hay-fever it should be understood that the pollens are the male elements of phaenogamous or flowering plants, and are, therefore, incapable of reproduction in the nostrils or tissues of hay-fever subjects, differing in this way from infection by bacterial micro-organisms. On this account, an artificially-produced

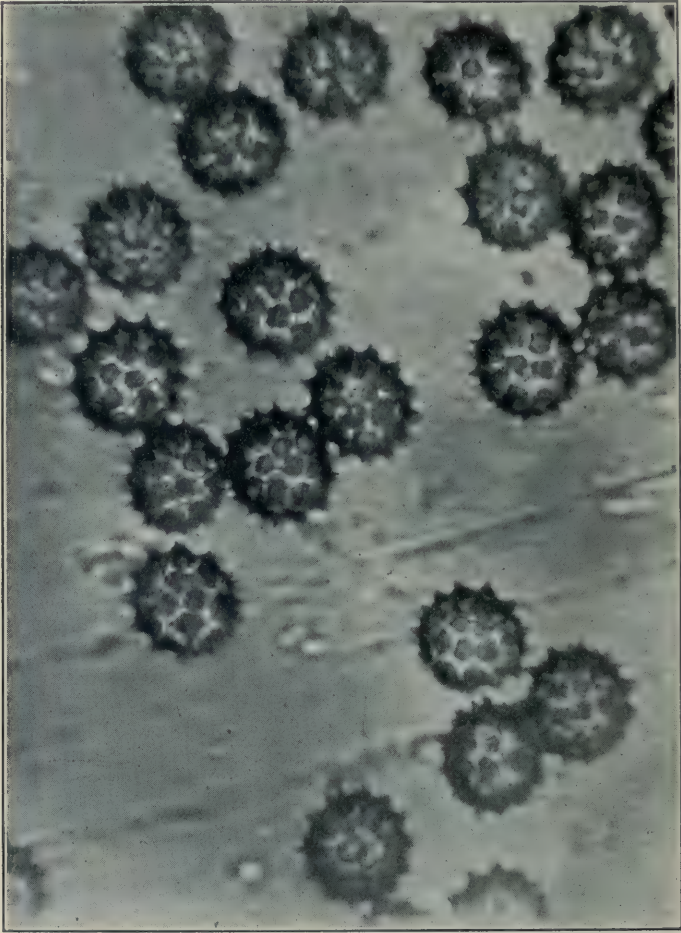


Fig. 1.—Pollen of bastard feverfew (*Parthenium hysterophorus*). The length of the spicules causes mechanical irritation of the nostril.  $\times 1,000$  diameters. (The photomicrographs illustrating this article were prepared in the biologic laboratory of the American Hay-Fever-Prevention Association.)

attack depends on the amount and strength of the pollens used or their extract, and, if the reaction is developed outside of the usual hay-fever season of the subject when there are no toxic pollens in the atmosphere, the length and degree of the attack may be accurately controlled.

This explanation is made in view of the repeated inquiry as to whether an artificially-produced reaction will not result in an attack of hay-fever of the usual duration. That this will not be the case will be understood when the nature of this process is explained.

#### REACTION FROM HAY-FEVER POLLENS

The reaction from hay-fever pollens may be divided into the direct and indirect effect. The former is due to the direct irritating effect of the pollen on the nasal mucosa. This may be mechanical, as with many

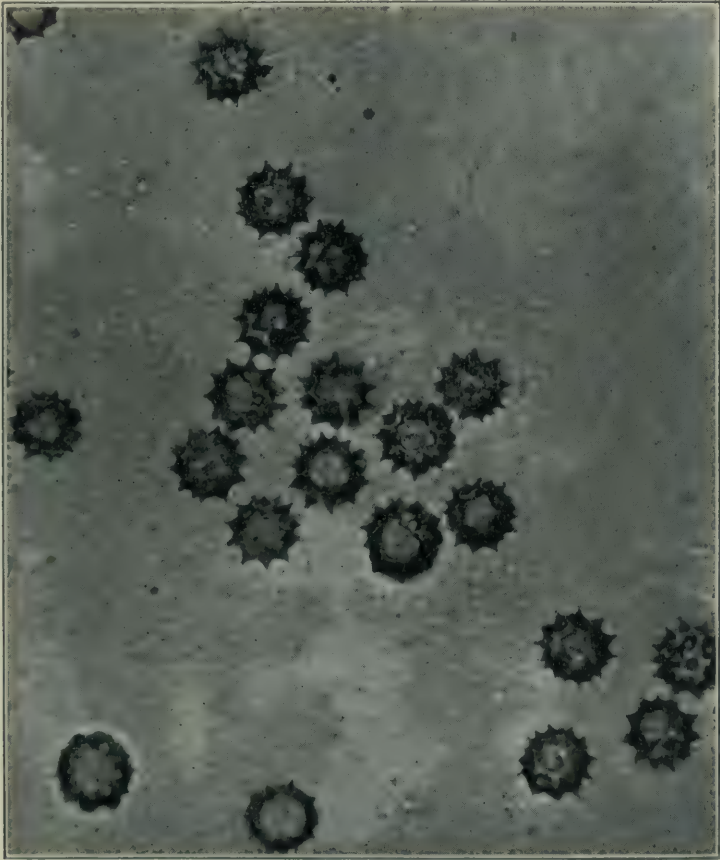


Fig. 2.—Pollen of sneezeweed (*Helenium quadridentata*). Also has marked spicules.  $\times 500$  diameters.

of the spiculated pollens. In some of these, as in the bastard feverfew (*Parthenium hysterophorus*, Fig. 1) and the sneeze weed (*Helenium quadridentata*, Fig. 2) the spicules are so prominent that they produce a reaction, due to the mechanical impact, even in persons who are not susceptible to hay-fever. This effect is immediate, and, in persons not

subject to hay-fever, is relieved as soon as the irritating pollens are discharged.

The primary effect may also be physiochemical in its character. In the wormwoods (*Artemisias*), which replace the ragweeds (*Ambrosias*) in the Rocky Mountain and Pacific states, the primary effect of the pollens, which are smooth, is as rapid and much more violent than with the spiculated pollens. The examination under the microscope shows that as soon as this variety of pollen comes in contact with the nasal secretion or the normal saline solution, it immediately ejects a portion of its contents from one or all of the three points of intersection of its three-lobed extine (Fig. 3). This fluid, which does not respond to the Lugol solution for starch, is a toxalbumin markedly irritating to the nasal mucosa, which explains the rapidity and extent of the local irritation.

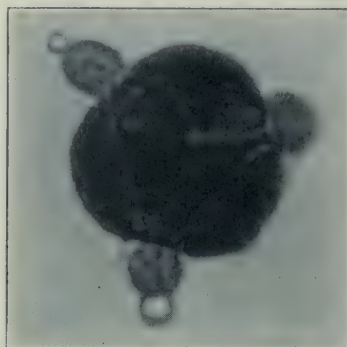


Fig. 3.—Pollen of wormwood (*Artemisia absinthium*). Showing effect of the absorption of nasal mucus.  $\times 1,000$  diameters.

The degree and duration of the primary effect of pollens is influenced by the irritability of the nasal mucosa of the patient. In some cases an intumescence of the nasal mucosa results which causes occlusion of the nostril and retention of the pollens, thus favoring absorption of their toxalbumins. In others, pollen causes a free discharge of a secretion which contains but few mucous corpuscles, but the usual salts of the nasal secretion (Fig. 4). The bacteriologic examination of a succession of hay-fever patients showed but slight variation from the bacteria found in the mucous secretion of the normal person. Pollen, however, is found in a large proportion of cases (Fig. 5) if the examination is made of the nasal discharge in the morning after the secretions have accumulated during the night.<sup>2</sup>

The indirect effect is due to the absorption of some portion of the pollen contents — the toxalbumins — under the influence of the nasal

2. Scheppegegrell, W.: Ragweed Pollen in the Nasal Secretion of Hay-Fever Cases, *Med. Rec.*, New York, October, 1916.





Fig. 4.—Nasal secretion of hay-fever patient showing preponderance of the salts and small number of mucous corpuscles.  $\times 125$  diameters.

secretion. This is demonstrated by the fact that the aqueous extract of toxic pollens produces a reaction in hay-fever subjects similar to the pollens themselves. This is characteristic of all hay-fever pollens. In the pollen of the *Gramineae*, in which the local (direct) reaction is slight, it is the principal cause of the hay-fever. Even in pollens in which the direct effect is marked (*Ambrosias*, *artemisia*) the absorption of the proteins is the important factor which prolongs the attack.



Fig. 5.—Pollen of common ragweed (*Ambrosia elatior*) in the nasal secretion of a hay-fever patient.  $\times 250$  diameters.

#### HAY-FEVER POLLENS

While pollens are generated by all the members of the phaenogamous plants, only those which are wind pollinated need be considered in connection with hay-fever. While there are many plants whose pollen may cause the hay-fever reaction when applied to the nostrils,

only pollens which float in the air and can reach the nostrils in the course of normal respiration are responsible for true hay-fever. In the selection of hay-fever plants it is, therefore, important first to decide that they are wind pollinated,<sup>3</sup> as the pollens of these plants only are found in the air.

As a rule, this may usually be inferred from the appearance of the plant. Attractive flowers and perfumes are characteristic of insect-pollinated plants and the presence of these is usually sufficient to eliminate them from the list of wind-pollinated plants, as in the case of the roses and golden rods.

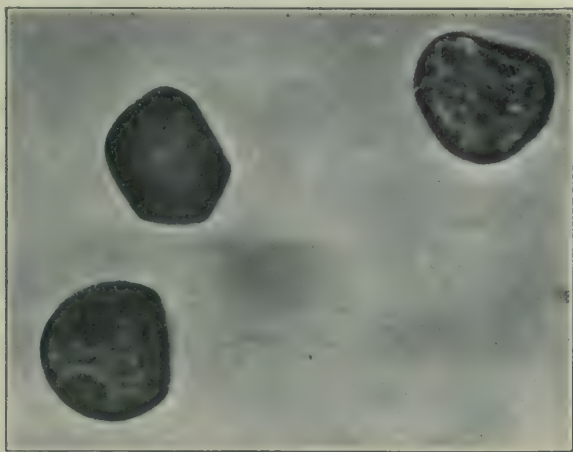


Fig. 6.—Pollen of plume grass (*Erianthus compactus*).  $\times 500$  diameters.

The absence of attractive flowers and perfumes is characteristic of the hay-fever weeds, as in the case of the ragweeds (*Ambrosias*), wormwoods (*Artemisias*), cockleburrs (*Xanthiums*), careless weeds (*Amaranths*), and the grasses (*Gramineae*).

The failure to limit the classification of hay-fever plants to those that are anemophilous, or wind pollinated, has resulted in the inclusion of many plants which are entirely harmless from a practical standpoint. In spite of the clearness of this principle, we are constantly receiving lists of hay-fever weeds from all parts of the United States which include the roses, golden rods, resin weeds and other insect-pollinated plants.<sup>4</sup>

3. The anemophilometer, an instrument for testing the wind-pollination of plants, has been devised by the Research Department of the American Hay-Fever-Prevention Association. William Scheppegrell, *Scientific American*, October, 1916.

4. Even in technical books published in the last two or three years, such plants as the evening primrose, chrysanthemum, aster, lily of the valley, honeysuckle and other insect-pollinated plants are described as furnishing the toxic pollens of hay-fever.



## THE POLLENS OF SPRING HAY-FEVER

From a test of many hundreds of pollens in our biological laboratory, the principal hay-fever plants have been selected. For the early type of hay-fever, the chief causes are the grasses (*Gramineae*), the special varieties varying according to climatic conditions (Fig. 6). This applies to all portions of the United States. The spring cases of hay-



Fig. 7.—Pollen of common ragweed (*Ambrosia elatior*). The principal cause of fall hay-fever.  $\times 500$  diameters.

fever are also influenced by other wind-pollinated plants such as the amaranths (*Amaranthus*), docks (*Rumex*), and chenopods (*Chenopodium*), which, however, are, in the aggregate, of much less importance both from the standpoint of reaction and geographical distribution.

## THE POLLENS OF FALL HAY-FEVER

The fall hay-fever, east of Kansas, is caused by the common ragweeds (*Ambrosia elatior*, Fig. 7), this being replaced in moist regions by the giant ragweed (*Ambrosia trifida*).<sup>5</sup> These cases are also affected by the pollens of the marsh elder (*Iva ciliata*) and cocklebur (*Xanthium canadensis*, Fig. 8), these, however, being of minor importance.

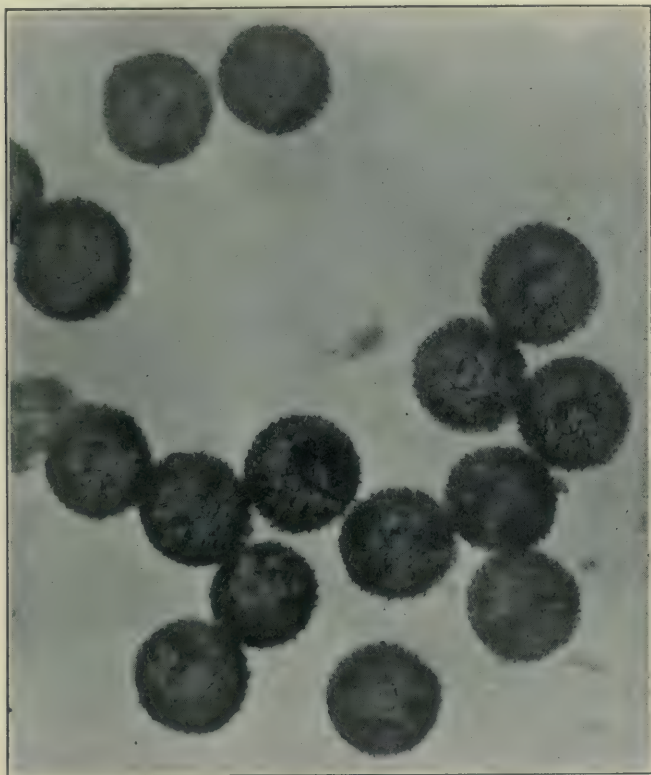


Fig. 8.—Pollen of cocklebur (*Xanthium canadensis*). A cause of fall hay-fever of minor importance, partly because it is less toxic than the common ragweed and also on account of its more restricted potential area.  $\times 500$  diameters.

In the Pacific and Rocky Mountain states, the ragweeds are replaced by the wormwoods (*Artemisias*, Fig. 9), the rough bur elder (*Iva xanthiifolia*), the *Gaertnerias* and the western ragweed (*Ambrosia psilostachya*).

## ATMOSPHERIC POLLEN PLATES

Atmospheric pollen plates are prepared by applying a thin layer of glycerin to the central square inch (25.4 mm.) of an ordinary microscope slide. We have tried various combinations but have found the

5. Scheppegrell, W.: Hay-Fever and Its Prevention, U. S. Pub. Health Rep., July 21, 1916, p. 1907.

pure glycerin, thoroughly applied to the glass which has been previously cleansed with alcohol, to be the most practical. In extremely moist weather, when the glycerin deliquesces too rapidly, we substitute a layer of boiled linseed oil.

We have tried various forms of apparatus for the purpose of having these plates always at right angles to the direction of the wind. While this furnishes useful statistical data, the complicated construc-

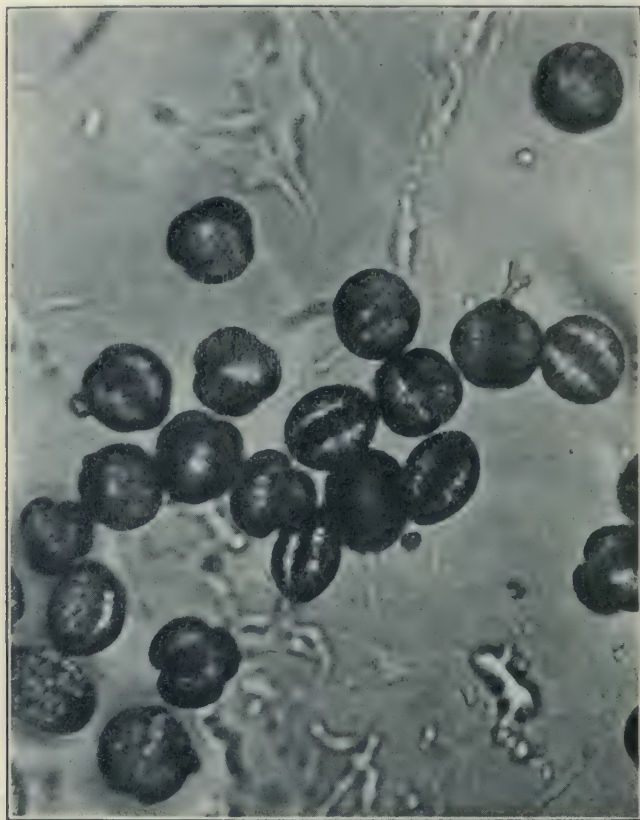


Fig. 9.—Pollen of wormwood (*Artemisia heterophylla*). One of the principal causes of fall hay-fever in the Pacific and Rocky Mountain States.  $\times 500$  diameters.

tion gives it a limited range. For practical purposes, the atmospheric pollen plates are simply exposed to the wind at the residence of the patient or at the special stations.

After twenty-four hours exposure, these plates are examined under the microscope, with the aid of the mechanical stage, so that the whole surface can be traversed. A low power (125 diameters) is sufficient for traversing the slide, and when a pollen grain is found, a high power (500 diameters) is used for its identification.



By the injection of a drop of Lugol's iodine solution, the count is simplified, as the grass pollens, which are stained blue-black, can be easily distinguished from other pollens which are usually stained brown.

After the pollens have been counted, they are reduced to the number per square centimeter, as our unit is the number of pollens per square centimeter for twenty-four hours.

As the object of the pollenometric records is to determine the amount of pollen in the air, a working formula has been prepared by means of which the number of pollen grains per cubic square yard of air may be determined from these plates.

#### NUMBER OF POLLEN IN THE AIR<sup>6</sup>

The number of pollen grains dropping on a given surface, for example, a plate, depends only on the number per unit volume of the air and velocity of fall of the pollen grains.

This means that unless the plate is inclined at an appreciable angle, for example, 15 or 20 degrees, with the direction of the wind near the plate, the number of pollen grains dropping on the plate is independent of the wind velocity, other factors, such as the number per unit volume of air, being constant. The number of pollen grains in the air, however, bears a direct relation to the velocity of the wind.

If the pollen grains fall with a velocity of  $v$  feet per second, and there are in the air  $n$  per cubic yard, the total number  $N$  falling on a square centimeter in  $t$  hours is given by the formula

$$N = 0.143 \times n \times v \times t$$

$$\text{and } n = \frac{7 \times N}{v \times t}$$

For example, if the diameter of the grain is 0.020 mm.,  $v = 0.16$  feet per second. If  $N'$  pollen are gathered in twenty-four hours

$$n = \frac{7 \times N}{0.16 \times 24} = 1.8 N'$$

If  $N'$  represent the number of pollen grains gathered per square centimeter on an atmospheric-pollen plate in twenty-four hours and the number of pollen per cubic yard is represented by  $n$ , the following are the numbers by which  $N'$  is to be multiplied to give  $n$ , for the pollen of different diameters:

Diameter = 10 microns,	$n = 7.3 N'$
Diameter = 15 microns,	$n = 3.2 N'$
Diameter = 20 microns,	$n = 1.8 N'$
Diameter = 25 microns,	$n = 1.2 N'$
Diameter = 30 microns,	$n = 0.8 N'$
Diameter = 40 microns,	$n = 0.45 N'$
Diameter = 50 microns,	$n = 0.30 N'$
Diameter = 60 microns,	$n = 0.20 N'$
Diameter = 70 microns,	$n = 0.15 N'$
Diameter = 80 microns,	$n = 0.12 N'$

To obtain the number of pollens per kilo-liter, divide the number  $N'$ , per cubic yard by 1.3.

6. Report of Prof. J. H. Clo, Department of Physics, Tulane University, New Orleans.

In October, 1916, the pollenometric records indicated nineteen common ragweed pollen grains for twenty-four hours. As this pollen has a diameter of 15 microns, we multiply the number of pollen by 3.2 of the formula, which shows that there were sixty-one pollen grains in each square yard of air on that date.

#### NASAL FUNCTION IN ARRESTING HAY-FEVER POLLENS

Several clinical tests were made in order to determine what percentage of atmospheric pollen grains are arrested in the nostrils. The conformation of the nasal passages is especially adapted not only for warming and moistening the inspired air, but also for arresting mechanical impurities, the tests showing that over 70 per cent. of the inhaled hay-fever pollen grains are arrested in the nasal passages.

Aug. 12, 1916, all of the nasal secretion of a hay-fever patient for eight hours was collected. As the patient slept in an open sleeping porch, the inhaled air contained the average number of aerial hay-fever pollens of that date and location. The number of pollen grains which the secretion contained were then counted and found to be 56. On the same date, the pollenometric record showed 10 giant ragweed (*Ambrosia trifida*) pollen grain for twenty-four hours. As these pollen grains have a diameter of 20 microns, the number per cubic yard was found to be 18 according to our table of atmospheric pollens. As the volume of air (tidal) for eight hours' respiration was 4.28 cubic yards, the patient had inhaled ( $4.28 \times 18$ ) 77 pollen grains during the eight hours. As 56 pollen grains were found in the secretion, it demonstrated that the nostrils had arrested about 73 per cent. of the grains which the patient had inhaled.

#### THE POLLENS OF HAY-FEVER VERIFIED BY THE ATMOSPHERIC POLLEN PLATES

The pollens responsible for hay-fever were verified by the atmospheric-pollen plates, which our research department exposed during the whole of the past hay-fever season. During the prevalence of the spring hay-fever, these plates, which were exposed at various stations and also near the residences of the hay-fever patients, invariably showed the grass pollens with a small percentage from other wind-pollinated plants such as the docks, amaranths and chenopods. The number of pollen grains deposited bore a definite relation to the intensity of the hay-fever attack.

In the fall hay-fever season the pollenometric records showed the presence of the ragweed almost exclusively (Fig. 10). August 15, the grasses had practically completed their pollination and their pollens had

almost disappeared from the air, as shown by these plates. The patients who were affected with the grass pollens had ceased to suffer from hay-fever, except those patients who were sensitive to both the grass and ragweed pollens.

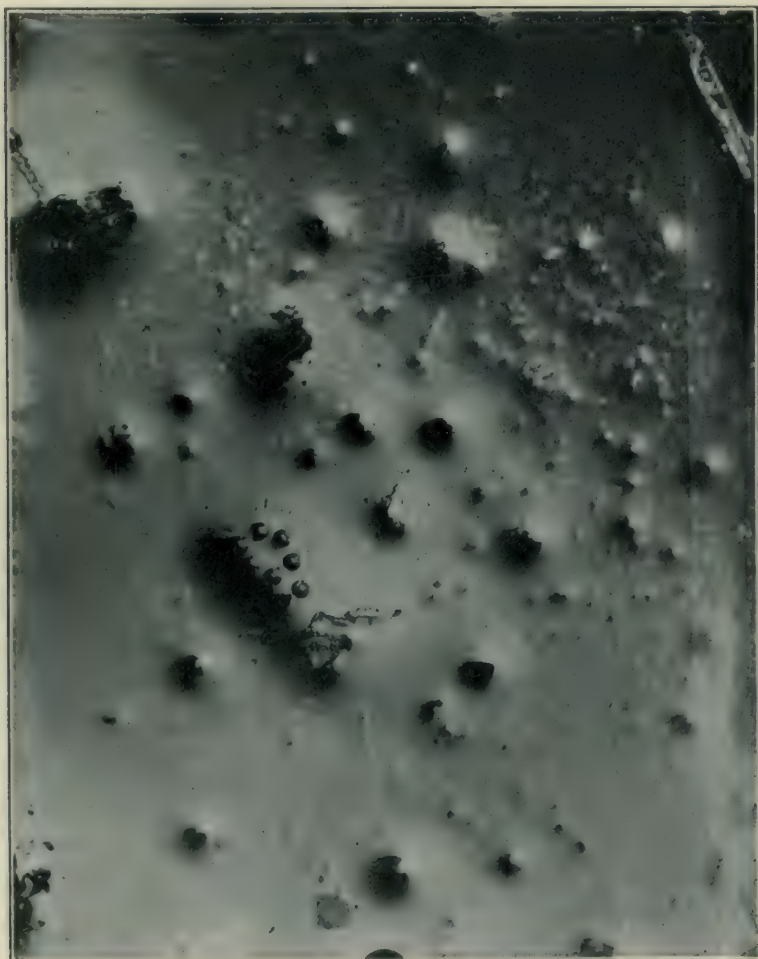


Fig. 10.—Atmospheric pollen plate showing prevalence of the common ragweed during the fall hay-fever season.  $\times 125$  diameters.

#### BUOYANCY OF THE RAGWEED POLLENS

Our pollenometric records demonstrated the remarkable buoyancy of the common ragweed pollen. In the vicinity of New Orleans, the common ragweed (*Ambrosia elatior*) is not found, being replaced by the giant ragweed (*Ambrosia trifida*), the nearest being about 5 miles from the station from which the important pollen records were made.



Nevertheless, during the prevalence of a 20-mile wind, the common rag-weed pollen grains were freely deposited on the plates located on the eighth floor of the Audubon Office Building of New Orleans.

On the other hand, the atmospheric pollen plates exposed at our Hendersonville (North Carolina) station (altitude 2,350 feet), Sept. 20, 1916, after the pollination of the common ragweed (*Ambrosia elatior*) had been ended in that section by the cold weather, gave negative

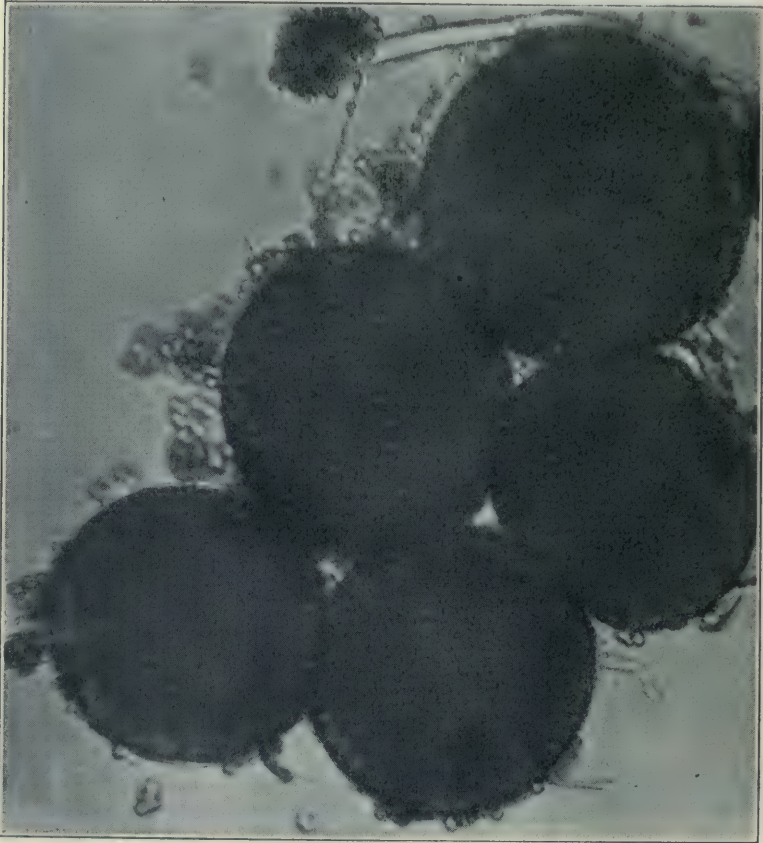


Fig. 11.—Pollen of corn. Its large size limits its potential area. This specimen is infected with the mycellium fungus to which the pollen of the *Gramineae* are susceptible.  $\times 500$  diameters.

results and hay-fever entirely disappeared, although at a distance of 15 miles the ragweeds were still pollinating profusely, with the usual number of hay-fever cases. While it has not yet been established to what distance the pollen grains of the common ragweed can be carried in sufficient numbers to cause hay-fever, our records for the past ten years show that it will not traverse this distance.

## POTENTIAL AREA OF HAY-FEVER POLLENS

As already stated, only the plants with wind-borne pollen need be considered etiologically in hay-fever, and of these, only those which have been shown to give the hay-fever reaction. The distance, however, at which the wind-borne pollens, giving a positive reaction, are responsible for hay-fever is influenced by their buoyancy, which controls the potential area of these pollens.

The buoyancy of pollens varies principally according to the size of the grains, this being also influenced by their external shape. The size of the hay-fever pollen grains varies within considerable limits, among the smallest being the common ragweed (Fig. 7), measuring 15 microns in diameter, and among the largest the pollen of the corn (80 microns, Fig. 11).

As the potential area is an important subject in the consideration of hay-fever, we have prepared a table by which this area for pollens of 10 to 100 microns in diameter, and with winds from 2 to 30 miles per hour, may be calculated.

CONVECTION OF HAY-FEVER POLLENS<sup>7</sup>

Any small particles which appear to float in the air, like a cloud of dust, fog, pollen, etc., are really going through a process of falling with a uniform velocity. The rate of falling is under these conditions independent of the density of the particle. If the surface of the particle is rough the velocity is less than if it is smooth.

If the particles have the form of smooth spheres of a diameter not less than 0.001 mm., it has been shown both on theory and by actual experiment that the velocity with which such spheres fall is given by the formula:

$$v = \frac{2 g r^2}{9 k} \quad (\text{Stokes' Law})$$

where  $v$  = the velocity in centimeters per second,  $g$  = 980 cm. the acceleration of gravity,  $r$  = the radius of the sphere and  $k$  = the coefficient of viscosity for air = 0.00018.

By substituting the different values for the radius of spheres and the known constants in the above formula, the velocity for pollen grains of any size can be calculated. The calculation gives exact values for the smooth pollen grains only. The values will be considerably higher in the case of the pollen with spicules, the velocity being considerably less than the calculated value.

When the velocity has been calculated, the time of fall of a pollen grain can be calculated for any distance by dividing the distance by the

7. Report by Prof. J. H. Clo, Department of Physics, Tulane University, New Orleans, and C. C. Clark, Acting Chief of Weather Bureau, Washington, D. C.

velocity. If a wind is carrying the pollen horizontally while it is thus falling, the distance it will be carried is equal to the product of the velocity of the wind times the time of fall. In this way the distance which grains of different size will be carried can be calculated.

The above formula has been used extensively in physical investigations, and there are numerous proofs of its correctness. The accepted value of the electric charge of an electron or cathode particle depends on the accuracy of this formula.

Table 1 shows the distances that pollen grains of various diameters will be carried by different winds from plants of different heights.

TABLE 1.—POTENTIAL RADII OF HAY-FEVER PLANTS  
Table of distances traversed by pollens of various sizes

Diameter in Microns	Velocity of Fall, Ft. Sec.	Distances in Feet Traversed by Pollen Blown from Plant 2½ Feet High Along Level Ground with Wind Velocity of							Distances in Feet Traversed by Pollen Blown from Plant 5 Feet High Along Level Ground with Wind Velocity of						
		2 Mi. Hr.	5 Mi. Hr.	10 Mi. Hr.	15 Mi. Hr.	20 Mi. Hr.	25 Mi. Hr.	30 Mi. Hr.	2 Mi. Hr.	5 Mi. Hr.	10 Mi. Hr.	15 Mi. Hr.	20 Mi. Hr.	25 Mi. Hr.	30 Mi. Hr.
10	0.04	184.0	460.0	920	1,380.0	1,840	2,300.0	1½ mi. 2,760	368	920	1,840	2,760	3,680	4,600	1 mi. 5,520
15	0.09	82.0	205.0	410	615.0	820	1,025.0	1,230	164	410	½ mi. 820	1,230	1½ mi. 1,620	2,030	0.45 mi. 2,460
20	0.16	46.0	115.0	230	385.0	460	575.0	690	92	230	460	690	920	1,150	1,280
30	0.36	20.0	51.0	102	153.0	204	255.0	306	40	102	204	306	408	510	602
40	0.64	11.0	28.5	57	85.5	114	142.5	171	22	57	114	171	228	285	342
50	1.00	7.5	18.5	37	55.5	74	92.5	111	15	37	74	111	148	185	222
60	1.44	5.0	12.5	25	37.5	51	62.5	76	10	25	50	75	102	127	152
70	1.96	4.0	10.0	19	29.0	38	50.0	58	8	19	38	57	77	96	116
80	2.56	3.0	7.0	14	21.0	28	35.0	43	6	14	28	42	56	70	86
90	3.24	2.0	5.5	11	16.5	22	27.5	33	4	11	22	33	44	55	66
100	4.00	2.0	5.0	9	14.0	18	25.0	27	4	9	18	27	36	45	54

NOTE.—For spiculated pollens, add 10 to 50 per cent., according to their size and the length of the spicules.

While Table 1 is correct for distances on level ground and under a steady wind of the given velocity, these exact conditions are not often met with. Such obstructions as houses, trees and shrubbery, will deflect the wind and cause the currents of air to go upward instead of horizontally. Hilly land also causes considerable variation in the effects of the wind, and also the vertical currents in summer. In spite of this, however, we have found this table of practical value as corroborated by our pollenometric records. It is especially useful in determining the relative distances traversed by various pollens. The common ragweed (*Ambrosia elatior*), for instance, having grains measuring 15 microns in diameter and spiculated, will traverse one-half mile in a wind of 20 miles per hour on level ground, and many times this dis-



tance when deflected by upward currents, while the smooth corn pollen will travel only 102 feet under the same conditions. Under similar conditions, the pollen grains of the grasses (*Gramineae*), which usually measure about 40 microns in diameter, will traverse 456 feet.

These calculations compare favorably with the records of our pollenometric records. The common rag-weed (*Ambrosia elatior*, with grains measuring 15 microns in diameter) will travel the greatest distance, which easily gives it the preponderance of hay-fever cases in the localities in which it is found.

The small number of cases of corn pollen hay-fever compared with that of grass pollen hay-fever is explained principally by the difference of size, causing a contracted radius of infection. In addition to this, corn is not found as a weed in the cities and suburbs, as we find the grasses and ragweeds, which, together with the limited carrying distance, explains the comparative rarity of corn pollen hay-fever.

#### NUMERICAL DISTRIBUTION OF HAY-FEVER POLLENS

In addition to the convection of pollen by the wind, we must consider the numerical distribution of these pollens. This is not subject to any special rule, as for the distance, and we must be guided by the pollenometric records as a basis of calculation.

These records show that this distribution is approximately inversely as the square of the distance. Theoretically, if the ground were level and the wind steady and from one direction, the distribution of the pollen would be inversely as the distance. On land, with its shrubbery, trees and buildings, the wind becomes a succession of wind currents, so that at one time the wind is at its full velocity, and at another may be almost still. During this interval, the pollen falls with a velocity shown in the table, and in this way a large portion is deposited on its way to the terminal distance based on the wind velocity. There are certain variations in the direction of the winds also, causing the pollen to be distributed in various directions. While, therefore, there is no exact rule for calculating the numerical distribution of the pollen, the rate established by our pollenometric records is approximately correct.

Some investigators have endeavored to calculate the amount of pollen generated by the ragweeds, and have estimated from this the amount of pollen in the atmosphere. As a matter of careful observation, however, over 50 per cent. of the pollen falls within the immediate neighborhood of these weeds. This is shown by the fact that when they grow in great abundance, the ground under them is colored yellow by the fallen pollen, this color rapidly diminishing a few yards from the plants. While, therefore, these pollens are distributed in great

numbers, the quantity in the air is really only a small proportion of the pollen generated.

The buoyancy of the common ragweed pollen can be better appreciated when it is stated that it requires 380,000,000 pollen grains to make 1 gm.

#### AMOUNT OF POLLEN DISTRIBUTED BY HAY-FEVER WEEDS

In order to ascertain the number of pollen distributed by hay-fever weeds, the following test was made June 24, 1916:

Six of the flowers of the bastard feverfew (*Parthenium hysterophorus*) while attached to the parent plant were enclosed in a small case, one end being left open to prevent the accumulation of moisture. At the end of four days the floor of the receptacle was found to be dusted with the pollen. To facilitate the counting, this area was divided into 110 squares and the pollen of one of the squares placed under the microscope. This gave a count of approximately 5,000 pollen grains for each square. As there were 110 squares, this showed that there were 550,000 pollen grains in the receptacle. Dividing this by 4 gave the number of pollen grains distributed by one flower in one day, this being 22,916.

An average large bush (3 feet, 10 inches in height) of the parthenium was then selected and the flowers counted, this being done by cutting off each flower. This count gave 9,902 flowers to the bush. Multiplying this by the number of pollen grains distributed by each flower, showed that the parthenium generates approximately 227,000,000 pollen grains per day.

The ragweeds, however, are much more prolific in their pollens, as shown in the following test: Aug. 23, 1916, a plant of the giant ragweed (*Ambrosia trifida*) was placed in such a position in the biological laboratory that all the pollen fell on the platform on which the plant was placed. The pollen which fell from 8 a. m. to 1 p. m. was divided into spaces and the pollen of one of these placed under the microscope and the grains counted. The total number calculated from these was found to be approximately 8,000,000,000. In this case there were only about thirty racemes pollinating, which is only a small number for this weed.

Similar experiments demonstrated that the number of pollen grains generated in a field of grasses (*Paspalum dilatatum*) during the active stage of pollination was about eight millions per square foot of surface. On account of the short distance from the ground (18 inches) and the large size of the grains (40 microns), only a small proportion of this pollen is carried into the air except during winds of high velocities (20 or more miles per hour).



An interesting point in connection with atmospheric pollen was demonstrated by these plates. A number of published reports show that atmospheric pollen came from the direction of the sea in which the nearest land was 200 miles, and it was, therefore, concluded that the pollen had traversed this great distance.

In New Orleans the ragweed area is northeast to northwest of the city, and under ordinary circumstances the pollen comes from this direction. Sept. 29, 1916, when the pollination of the ragweed was at its height in this locality, the wind, which was from the northeast, rose to 22 miles per hour and the pollen count reached 114. Two days afterward, the wind veered to the southeast, in which direction but little ragweed is found. The pollen plates, however, continued to show nineteen pollen grains for two days afterwards. This shows that the high wind had infected the atmosphere with the ragweed pollen for so many miles and to such an altitude, that for several days it continued to descend from the direction in which it had been carried. Had the initial record of the northeast wind not been made, it would have been supposed that the pollen had originated from some southern point of great distance.

#### WINDS IN RELATION TO HAY-FEVER

It is well known that hay-fever patients improve during a rain of several days' duration, and that their symptoms become aggravated during the prevalence of high winds. The former is due to the fact that pollens floating in the atmosphere are precipitated by the rain and that no more pollen rises until it ceases. This has been clearly shown by our pollenometric records. During high winds, on the other hand, not only are more pollen grains shaken from the plants, but the liberated grains are carried to proportionately greater distances.

These records also demonstrate that there is a definite relation between the wind velocities and the amount of pollen in the air, the clinical reports proving that there is a similar relation between the resulting pollen in the air and the symptoms of hay-fever subjects.

Table 2, which is a partial report of one of our stations, shows the records of the atmospheric pollen plates in relation to the daily maximum and mean wind velocities and directions, the estimated number of pollen grains per cubic yard of air, and the mean temperatures and the rainfall. As this station is at an elevation of 100 feet (eighth floor of office building) and at least 2 miles from any large weed areas, the records are uninfluenced by any special local conditions. The table gives the number of grass pollens, of ambrosia pollens (common and giant ragweed) and of "other pollens," the latter including the docks, amaranths, chenopods, marsh elders and cockleburrs, all of which give a positive reaction for hay-fever, but are in this locality of minor importance.



TABLE 2.—HAY-FEVER POLLENS IN RELATION TO WIND, TEMPERATURE AND RAIN

	Grass Pollens	Ambro- sia Pollens	Other Pollens	Number of Pol- lens Per Square Yard of Air	Maxi- mum Wind	Mean Wind	Direc- tion	Mean Temper- ature	Rain
1916									
9/22	4	5	2	29	10	3.8	N.W. S.	78	0.0
9/25	..	7	..	22	14	5.4	S.E. S.	80	0.0
9/26	1	9	..	31	16	6.0	S.E. S.	80	0.01
9/28	..	15	..	48	15	4.3	N.E. S.	82	0.02
9/29	..	114	..	365	22	13.4	N.E. N.E.	68	0.0
10/ 1	..	36	..	115	16	7.5	E. S.E.	68	0.0
10/ 2	..	19	..	61	14	7.4	E.	71	0.0
10/ 3	..	5	..	19	12	5.9	E. N.E.	72	0.0
10/ 4	..	7	..	22	12	6.2	E.	74	0.0
10/ 5	..	11	..	35	21	10.7	N.E. N.E.	73	0.01
10/ 6	2	6	2	29	16	7.2	E. E.	78	0.01
10/ 7	1	7	..	26	17	7.9	N.E. N.	76	0.02
10/ 8	..	2	..	6	12	4.3	S.E. N.W.	80	1.85
10/ 9	..	8	..	26	7	2.9	S.	80	0.0
10/10	..	12	1	42	18	9.6	N.E.	74	0.0
10/11	..	11	..	35	18	7.7	N.E. E.	73	0.0
10/13	..	3	1	13	7	4.3	S.E. S.E.	77	0.0
10/14	..	9	2	35	10	5.0	S.	78	0.0
10/17	..	..	..	0	25	14.0	E. N.E.	72	2.58
10/18	..	1	4	16	24	11.5	S.W. N.E.	74	0.19
10/19	..	23	5	90	15	7.9	S.W.	76	0.0
10/20	..	5	5	32	23	13.4	N.W.	62	0.0
10/21	..	12	2	45	15	8.5	N.W.	58	0.0
10/24	..	..	2	6	8	4.2	E.	67	0.0
10/25	..	1	1	6	15	7.1	N.W.	70	0.0
10/26	..	8	2	32	17	10.1	N.E.	62	0.0
10/27	..	1	3	13	15	8.5	N.E. N.E.	66	0.0
10/28	..	1	8	29	12	6.5	E. N.W.	67	0.0
10/29	..	..	1	3	9	4.5	N. N.	70	0.0
10/31	..	..	2	6	8	3.7	N.W.	72	0.0

Our clinical records show that the most severe symptoms of fall hay-fever were on September 29, 30, 31 and October 1, which corresponds with the high wave of the recorded pollen count.

After the nostrils have become irritated by a large number of pollen grains they become sensitive to a smaller number than under ordinary conditions. Thus, each period of aggravated hay-fever was followed by symptoms in excess of the record of the pollen counts.

As will be noted, these counts are influenced not only by the wind velocity, but also by its direction, this being regulated by the locality of the nearest large weed areas. September 22, there were 11 pollen grains per square centimeter with a wind of 10 miles from the northwest, while on September 25 there were only 7 with a wind of 14 miles from the southeast.

Towards the latter part of October the pollination of the ragweed was reaching its limit, which explains the irregular count after October 15. October 29, the ragweed pollens were no longer recorded on our plates, and our clinical records show that the fall hay-fever season has passed.

The effect of rain on the pollen was also clearly indicated. October 17, the rain of 2.58 inches caused the ragweed pollen to disappear from the plates during that day, and also the following (1 per sq. cm.) in spite of the fact that the wind had reached a velocity of twenty-four miles per hours and was from a northerly direction. Local showers have little effect, as shown on September 26 and 28, and October 5, 6 and 7.

#### INFLUENCE OF TEMPERATURE

It has been frequently reported that hay-fever is aggravated by hot weather, but the records for the past year indicate that high temperatures have no special effect. On the contrary, the records both pollenometric and clinical show that most of the severe stages of hay-fever developed on days when the temperature was unusually low for the season. Thus, the most severe stage of hay-fever during the past season in New Orleans was on September 29 and 30, when the mean daily temperature was 68, the normal for this being about 80. The lower temperature, however, was only incidental, being due to the high wind (22 miles per hour) which blew from the northeast, which in this case was also from the direction of the hay-fever area.

#### EFFECTS OF PREVENTIVE MEASURES AGAINST HAY-FEVER POLLENS

Our pollenometric records during the past year demonstrate that the vernal type of hay-fever is due principally to the pollen of the grasses, and that these are not carried a long distance by the wind. In neighborhoods in which the weeds are systematically cut, the pollen count is very low and the cases of hay-fever unusual. In other localities, in which the weeds have been neglected, the pollen count is high and cases of hay-fever common.

These records have also demonstrated very clearly that the vernal form of hay-fever may be practically eradicated by the enforcement of adequate grassweeds ordinances. In New Orleans, where, through the efforts of the local committee of the American Hay-Fever-Prevention

Association, cooperating with the Department of Public Property and the Board of Health, the grassweeds ordinance has been thoroughly enforced, vernal hay-fever has almost disappeared.

In the fall type of hay-fever, however, the results have not been so marked. The buoyancy of the pollen of the common ragweed (*Ambrosia elatior*) is such that it will travel several miles in winds of high velocity (15 to 20 miles per hour). In view of this, municipal efforts alone will not be able to eradicate fall hay-fever, although much benefit may be obtained. Complete relief will follow only when the public realizes the great benefit which will result from the eradication of the worthless ragweed that forms the principal cause, and state legislation reinforces municipal efforts in its eradication.

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# THE SYSTOLIC BLOOD PRESSURE FOLLOWING EXERCISE; WITH REMARKS ON CARDIAC CAPACITY \*

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The observations related in this article were undertaken after reading the recent articles published in this journal by Barringer<sup>1</sup> in which he describes a method used by him to estimate the capacity of the heart in normal and diseased subjects. The work has been done at the Hampstead Military Hospital, London, which institution has been set aside by the British War Office for the investigation and treatment of soldiers suffering from cardiac disabilities. After reading Barringer's article it seemed probable that we might find some qualitative difference between the blood pressure reaction following given test exercises, in healthy subjects and in those patients who form the greatest part of our material, namely, soldiers who suffer from the condition described as "irritable heart"; and that if such a difference could be discovered it would form a valuable help in sorting our patients for treatment. We have completed this comparison and have been unable to find the qualitative difference for which we looked, but in completing it we have acquired knowledge of the blood pressure reaction, which, so it seems to us, is at variance with the conclusions of Barringer. At present we publish such of our results as are relevant to a discussion of Barringer's hypothesis; confining the observations to the normal curve of systolic blood pressure which follows immediately on a complete test exercise.

*Barringer's Test.*—The subject performs a given amount of exercise. In some cases this is done by means of a Zuntz ergometer, in others by raising 5- or 10-pound dumb-bells from the shoulders, in others where larger amounts of work in a short space of time are

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\* From the Hampstead Military Hospital, London. Done under the terms of a Moseley Traveling Fellowship from Harvard University. This work was done for the Medical Research Committee at the direction of Dr. Thomas Lewis, and in cooperation with him and Capt. T. F. Cotton, C.A.M.C., to both of whom the writer acknowledges his indebtedness.

1. Barringer, T. B., Jr., and Teschner, J.: The Treatment of Cardiac Insufficiency by a New Method of Dumb-Bells and Bars. The Circulatory Reaction to Exercise as a Test of the Heart's Functional Capacity. *THE ARCHIVES INT. MED.*, 1915, **16**, 795. Barringer, T. B. Jr.; The Circulatory Reaction to Graduated Work as a Test of the Heart's Functional Capacity, *Ibid.*, 1916, **17**, 363; Studies of the Heart's Functional Capacity as Estimated by the Circulatory Reaction to Graduated Work, *Ibid.*, 1916, **17**, 670.

required, by swinging dumb-bells or a 25-pound bar from the floor above the head and back again. The time consumed in each form of exercise varies between thirty and 120 seconds.

For purposes of illustration we shall confine ourselves to the third form of exercise used by Barringer. His readings were taken at intervals with a Riva-Rocci manometer by the auscultatory method. We gather from his description that the first reading was obtained thirty seconds or less after the cessation of exercise. The actual moment is not stated, but the readings as charted, are placed on this time line. Succeeding readings were taken at one-minute (in some cases half-minute) intervals after the first. According to Barringer the first reading is higher than the second, indicating an initial fall of blood pressure, in healthy subjects who have undertaken moderate amounts of work. But in normal people after a full effort the second reading is the higher, indicating an initial rise of blood pressure which he names the "delayed rise." The occurrence of this rise is an index, according to this writer, that the full "cardiac capacity" has been reached, and the amount of work required to produce it is taken, both in normal and also in diseased subjects, as a measure of the functional capacity of the heart. We are led to understand that when the heart is submitted to a given amount of work, a qualitative difference appears in the succeeding curve of blood pressure; and it is to be emphasized that it is in that portion of the curve which is described during the first minute, or at the most the first minute and a half, of rest after exercise that this change is manifested; a change which is taken to indicate a "functional insufficiency" for the particular amount of work accomplished. The amount of work necessary to produce such insufficiency varies with the capacity of the heart, and is far greater in the normal subject than in those in whom the muscle is weakened by disease. Somewhat similar observations have been made by Gräupner,<sup>2</sup> whose work prompted that of Barringer; but Gräupner's first readings suffer from a similar defect to those of Barringer, in that their precise relation to exercise in point of time is not known; apparently it was not estimated.

From his experiments, Gräupner draws certain conclusions, namely:

1. Moderate amounts of work in strong persons produces, generally speaking, no changes in blood pressure (systolic) after work.
2. An increase in the work done results in the blood pressure standing higher immediately after work, then more or less quickly returning to normal.
3. If the work done is still more increased, we find a sinking of blood pressure immediately after the exercise. However, the blood pressure quickly rises above normal and then falls back to normal . . . As long as this secondary rise above

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2. Gräupner: *Deutsch. med. Wchnschr.*, 1906, No. 26. Gräupner and Siegel: *Ztschr. f. exper. Path. u. Therap.*, 1906, **3**, 109.

normal is present "functional insufficiency" exists . . . The weaker the myocardium, the less the amount of work necessary to produce "functional insufficiency."

4. If primary myocardial weakness is present, we speak of "pathological insufficiency." This is characterized by primary sinking of the blood pressure below normal, gradually rising to normal.

*Method.*—In the early experiments which we instituted, the conditions approximated as closely as possible to those described in Barringer's papers. We soon found that only rarely could the first readings be obtained earlier than twenty to twenty-five seconds after the cessation of work. On one occasion, however, after a subject had performed a small amount of work which there was no clinical reason to believe approached the limits of his capacity, and following which he showed no signs of distress, a reading was obtained within fifteen seconds. This reading was followed, as shown in successive readings, by a rise in systolic pressure.

This seemed to indicate the advisability of using a method which would enable us to obtain constant early readings.

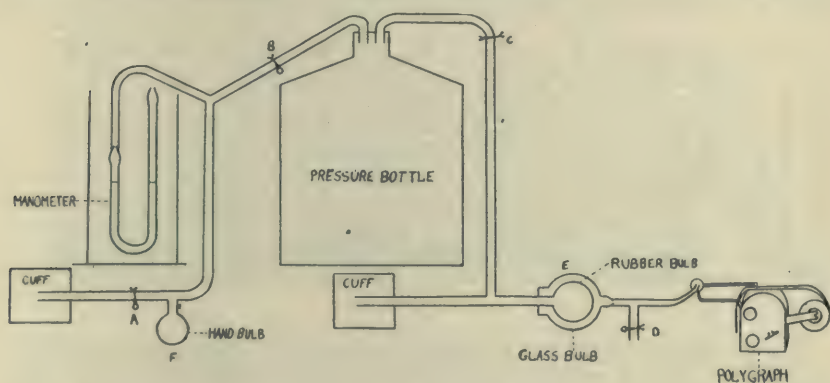


Fig. 1.—Apparatus used in Author's experiments, more fully described in the text.

A large bottle is connected (Fig. 1) by means of one system of rubber tubing with an arm cuff and a mercury manometer, so that when cock A is open and cock B closed this portion is an ordinary Riva-Rocci instrument. By means of the other system of tubing the bottle is connected with a cuff lying on the other arm, and through the bulb E (a rubber bulb open at the proximal end, inside of a glass bulb open at the distal end, such as is used in the Erlanger sphygmomanometer) to the writing lever of a Mackenzie ink polygraph.

Before the exercise the pressure bottle is filled with air at the desired pressure by the hand bulb (F) after closing cocks A and C and opening cock B. The amount of pressure used is such as has been shown in the given case to release into the two cuffs a pressure slightly below the subject's normal systolic pressure. Cocks B and C are closed, and cocks A and D opened. The subject then does a definite amount of work. Immediately before the cessation of work cocks B and C are opened, releasing the desired pressure into each cuff. The subject sits down at the end of exercise, cock D is immediately closed and the pulse at once recorded by the polygraph.



When the subject resumes the sitting posture, the cuff on the other arm, that is, that used for blood pressure determination, is already filled at a convenient pressure and only one or more pumps of the hand bulb are necessary to raise it above systolic pressure and to obliterate the pulse. By using this simple device we obtain (1) a continuous curve of pulse rate almost from the instant exercise ceases—these pulse rates will not be discussed in the present communication—and (2) a reading of systolic pressure, always within ten seconds, often within four or five seconds of the termination of exercise. This reading and the following readings are written on the pulse curve and their time relations subsequently determined accurately. As soon as the first pulse beats have forced the armlet the cuff pressure is raised a few millimeters until the beats again force their way through, and so the pressure curve is followed for from one-half to one minute by readings taken in rapid succession (at about five-second intervals) until a maximal point is reached; the cuff pressure is then released, and the remaining readings are taken more leisurely, the cuff pressure being reduced between each.

Barringer has used the auscultatory method of taking blood pressures, and criticizes the palpatory method. Both are, indeed, subjective methods, and facility in the use of either is a matter of practice. The supposed greater accuracy of the auscultatory method is unproved. Assuming a difference to exist, it is very slight; we have rarely found it to be more than 2 to 4 millimeters. The great advantage of the palpatory method is its quickness in obtaining early readings. We eliminate error so far as possible by repeating each observation

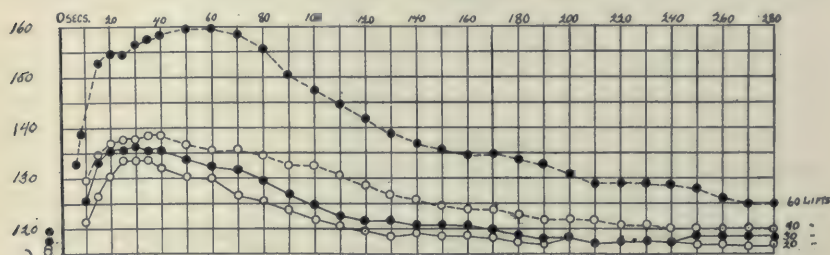


Fig. 2.—Weight of subject, 140 pounds; lift, two 10-pound dumbbells through 6 feet; time, 1 lift in 2 seconds. With 30 lifts, slight breathlessness; with 40 lifts, moderate breathlessness; with 60 lifts, considerable breathlessness and slight distress. As in all curves, the zero mark represents the cessation of exercise, the readings charted to the left of this line being controls taken before exercise.

on three successive days, plotting all three curves and taking the average of the three. The curves here published are averages of this kind.

We cannot agree that the continuous pressure of the cuff for a minute or less on the arm influences the readings in any appreciable degree. Before each observation the subject rests until constant and normal blood pressures and pulse rate are reached; these control observations are taken in the same fashion as those above described and the effect of cuff pressure on blood pressure thereby eliminated. The average of the three sets of control readings is given in conjunction with each curve published.

The exercises used are the swinging of two 10-pound dumbbells from the floor to above the head, the number of lifts varying from ten to sixty, the rate of lifting varying between 1 lift in 2, 1 lift in 3, and 1 lift in 4 seconds (guided by metronome). Not more than three different exercises are performed by the same subject in one day, and full rest is given between each. The possible influence of the effects of one exercise on another are eliminated

in this fashion and by varying the order of a given set of exercises on succeeding days.

*The Blood Pressure Curve.*—A young and healthy adult accomplishes 20 lifts at the rate of 1 lift in 2 seconds without breathlessness or subsequent fatigue; at the same rate he can accomplish 60 lifts more or less at the expense of some breathlessness and a varying amount of subsequent fatigue. But whether the

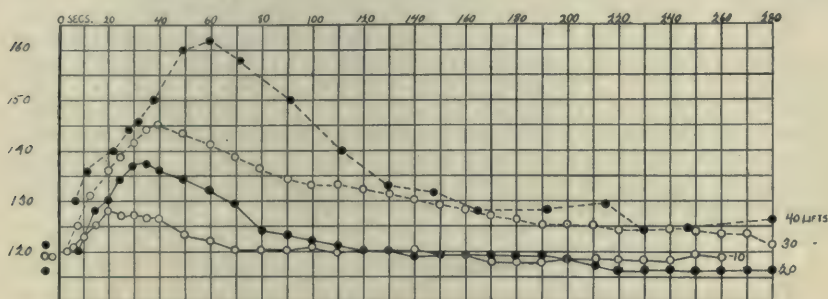


Fig. 3.—Weight of subject, 154 pounds; lift, two 10-pound dumbbells through 5 feet 10 inches; time 1 lift in 2 seconds. With 20 lifts, no breathlessness; with 30 lifts, moderate breathlessness and slight distress; with 40 lifts, considerable breathlessness and distress.

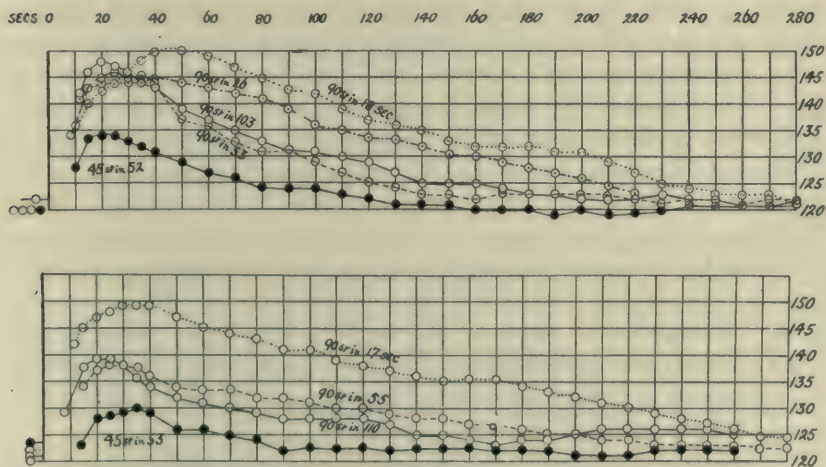


Fig. 4.—These two curves are the records of blood pressure following stairs climbing in normal subjects. The rate varied from a leisurely walk to a half run; the first producing in both cases practically no symptoms, the last resulting in considerable breathlessness, with gradations between the two. In the experiments, the results of which are figured in black, half as many stairs were climbed as in the other exercises. Each stair was 9 inches high.

lifts are 20, 30, 40, or 60, the reaction is qualitatively the same. The earliest readings show a prominent *rise* of blood pressure. An example of four reactions (each the average of three complete observations) is shown in Figure 2, and it may be taken as in most respects characteristic. The chart shows the after effects of 20, 30, 40, and 60 lifts (1 lift in 2 seconds) on the systolic blood



pressure. The control readings lie between 115 and 120 mm. Hg. The readings taken within the first ten seconds lie between 122 and 133 mm. Hg. From these points the curves sweep upward, reaching summits varying between 134 and 160 mm. Hg. Providing there is any reaction, *the rise is always present whatever the amount of work*. In this instance, as in most though not all instances, the greater the amount of work the higher is the first reading. In this, as in most instances, the amount of the rise varies directly with the amount of work.<sup>3</sup>

In the second chart (Fig. 3) the gradation is remarkably uniform.

Another feature which almost all these serial curves from normal subjects display, perfectly or in part, is the prolongation of the rise with increasing increments of work. The summit with different amounts of work is reached within 20 to 60 seconds; beyond 60 seconds we have not seen it prolonged. Beyond these points the curves fall gradually to the normal levels; they reach the normal line in times which vary with the amount of work; the fall is complete within 1½ or 2 minutes on the less severe exercises; and in 4 minutes, or perhaps a little longer, with the most severe exercises.

#### DISCUSSION

The conclusion which we draw from our observations is that as an immediate sequence of accomplished exercise, whether that exercise is moderate in degree, or whether it calls forth a full effort on the part of the person<sup>4</sup> who performs it, there is a rise of systolic blood pressure. The curve rises from a little above the original normal level to a point far above it; the rise is abrupt and has largely escaped detection on this account. This rise, in cases in which it is discovered, is spoken of by Barringer as the "delayed rise," and by him is considered to show that the exercise which produces it has overloaded the heart. With this conclusion we naturally disagree, but comprehend the manner in which he has been led to it. In most instances of moderate effort the whole of the rise to its summit will have been completed before Barringer obtains his first reading. In instances of great effort the rise will often be prolonged sufficiently for the first reading to be taken *on the rise*; the second reading will then be taken on the fall, or possibly on the continuation of the rise. In the first eventuality the difference in the readings will often be inconspicuous, that is, such few millimeters of mercury as are given in most of Barringer's tables;<sup>5</sup> in the second eventuality the rise will be conspicuous.

To speak of the rise ("delayed rise") itself as an index of a change in the circulatory reaction is, in the light of our experiences, unsound; to speak of a delay in the full development of the rise with severe effort, is usually to speak correctly. Even if it be admitted that this

3. Exceptions are found where two or more curves of such a series fall almost together.

4. We refer deliberately to the effort of the person and not to the effort of the heart, but we do not wish thereby to infer that the amount of work was limited by muscular fatigue.

5. The delayed rise as tabulated by Barringer is frequently no more than 2 mm. Hg. a difference which in our opinion is well within the error of measurement, however such readings are taken.



delay is of value, Barringer's method of determining it is too precarious to be serviceable. As the first reading is taken a little earlier or a little later, or as the second reading is taken a little later or a little earlier, so a fall becomes a rise, or a rise a fall. We regard this method of ascertaining *the shape of the blood pressure curve* to be without value, on account of the large variations in pressure which occur in the fraction of a minute following accomplished exercise.

But when we turn to the general thesis that such curves, even if *properly determined*, are indexes of the capacity of the heart, criticism is on broader lines. Briefly, we think that it is to be deplored that such theses are put forward in the present state of our knowledge. In so postulating a test of capacity Barringer does not stand alone, but in company with an increasing body of workers among whom little or no agreement of thought or methods is to be discovered.

#### THE USE OF THE PHRASES CARDIAC CAPACITY, CARDIAC EFFICIENCY

In speaking of a reservoir as capacious, an adjective is employed which in common parlance is convenient enough; but convenience renders the qualification no more exact. In dealing with matters practical, to state that a reservoir has a large capacity is insufficient; the statement of content is made in terms of the measure employed in gauging such capacity; the capacity is so many cubic feet, more or less. It is a truism that if the amount or size of a thing cannot be stated in terms of measure it cannot be stated at all; and however convenient it may be in conversation to refer to the size of a given object in general terms, such a device is frankly time and labor saving, or else it is deceptive.

It is common to see the phrase "capacity of the heart" employed, it is less common to see this phrase defined; but it may be presumed that it conveys the meaning "capacity to do work." Now the amount of work which can be done by an organ such as the heart, may or may not be measurable. If measurable in terms of foot-pounds per second, we are justified in speaking of cardiac capacity in this sense, adding the precise number of foot-pounds of work per second of which the heart is capable. If not measurable in these or some other units, the term cardiac capacity becomes a meaningless or misleading phrase. The work done by the heart in animal experiment and under given circumstances has been differently computed by various workers; such estimates are recognized as approximations subject to further revision. The work done by the heart in the intact and sentient animal at rest has never been accurately computed; still less has the *capacity* of the heart for work in circumstance of overload. It has become fashionable, nevertheless, to express the capacity of the heart for work in

terms of measure, terms which do not measure that capacity, but something which is quite different. Perhaps the pressure in millimeters of mercury necessary to collapse the brachial artery at a given moment and in a given condition of the body, or a series of such readings, is adopted. Perhaps similar readings of pulse rate are taken. The first is allowed as a measure by no means perfect of systolic blood pressure; the latter is allowed in most circumstances as a measure of ventricular rate. But they are no more than that. A curve of systolic blood pressure during or following exercise may be an exact expression of real events; but these events are blood pressure events and the measure is of blood pressure and not of cardiac work. It may prove that a certain form of blood pressure curve following a given exercise is found only in those who manifest grave signs of heart disease, and in whom the life history is to be short. We gain no advantage by concluding on anything but fully sufficient grounds that inefficiency or depressed cardiac capacity is responsible for the peculiarity of the curve on the one hand, the short life history on the other; we lose an advantage if thereby our vision of the real issue is clouded. The curve of blood pressure in question may be in our experience a sign that life is precarious, or that certain therapeutic measures may be adopted to the benefit of the subject. If this or that is the ascertained fact, it should suffice; it can be utilized to serve our ends just as satisfactorily without the interposition of hypothesis. The moment that the term "cardiac capacity" is introduced, though it may be introduced simply and solely to describe a given phenomenon of blood pressure or a series of such phenomena, additional conceptions of the heart as a whole or of the heart muscle in particular (histologic condition of the myocardium, reserve power of the muscle, etc.) are awakened, conceptions which as likely as not are unjustified, conceptions which in the minds of few workers will be identical.

When we are in the presence of physical signs and symptoms which unequivocally display slackening of the circulation, and when other physical signs point with equal clearness to *disease* of the heart as the central defect in the patient, then we may be justified in referring to cardiac inefficiency in summing up the condition; we *may* be justified also in conceiving a lowered cardiac capacity for work. Yet even in these circumstances we are often unable positively to assert that the heart is incapable of a normal amount of work; for though the circulation is failing, yet the heart may be overburdened with work (as a result of high peripheral resistance, extreme valvular obstruction, or the mechanical disadvantage of dilatation); and often, for aught we know, the work accomplished by a heart where circulatory failure is

manifest, is greater than the work to which a healthy organ could submit. The crux is that we have no measure of one or the other.

Admittedly the present argument applies with least force to such patients; but it is not in them that isolated physical signs are used as dependable; it is in the borderline case, or the case in which there are no other definite signs of circulatory failure, that they are employed, and employed, so it seems to us, often with insufficient justification.



## A NEW INTERPRETATION OF THE PATHOLOGIC HISTOLOGY OF HODGKIN'S DISEASE \*

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NEW YORK

There are two outstanding theories concerning the nature and origin of Hodgkin's disease: one, that it is primarily an infective granulomatous lesion of the lymph nodes with secondary changes in the spleen, liver and other tissues; the second theory postulates that the disease is neoplastic from the beginning. Neither offers an acceptable explanation of the process as a whole, and there is considerable evidence in contradiction of both.

The histologic changes in Hodgkin's disease were originally described by Sternberg<sup>1</sup> as the expression of a peculiar type of lymph node tuberculosis. Repeated efforts to detect tubercle bacilli in suitably stained microscopic preparations, and the injection of freshly emulsified lymph nodes into susceptible animals, have failed, however, to confirm this view, and it has since been generally abandoned. More recent efforts to implicate a diphtheroid micro-organism as the cause of Hodgkin's disease have likewise been discredited. Nevertheless, the prevalent opinion seems to be that the process is inflammatory, the neoplastic theory having failed of popular acceptance largely because the histologic changes in the lymph nodes and elsewhere are more in consonance with the conception of a granuloma. As a matter of fact, Hodgkin's disease appears to me to exemplify the frequently neglected fact that inflammation and neoplasia share several important features in common, and that the distinction between the two is, at best, academic and artificial, and, at times, impossible. Thus, the histologic changes in Hodgkin's disease bear a resemblance to those of tuberculosis on the one hand, and to a malignant connective tissue tumor on the other, while the distribution of the lesions in the liver, kidney, bone marrow, and other tissues not commonly included in the lymphoid system, is strikingly like that of a metastasizing tumor, but is also explicable on the basis of inflammatory hyperplasia of pre-existing lymphomatous foci. There is still another possibility, however, which does not appear to have been previously emphasized, namely, that the histologic changes in the lymph nodes, spleen and other parts of the

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\* From the Laboratories of Pathology, Bellevue and Allied Hospitals, Director: Dr. Charles Norris.

1. Sternberg: *Centralbl. f. d. Grenzgeb. d. Med. u. Chir.*, 1899, **2**, 641.

lymphoid apparatus in Hodgkin's disease constitute a systemic process with multiple foci of disease arising in different localities at approximately the same time and in response to the same provocative agent. In other words, the fully developed Hodgkin's disease, although possessing a histology all its own and maintaining in this regard an unmistakable individuality, nevertheless partakes of the nature of an infective granuloma on the one side, and of systemic lymphosarcomatosis on the other — that is to say, the disease is neither a pure granuloma nor a neoplasm, but a lesion belonging to a group for which a descriptive title has not yet been formulated, and which may, with propriety, be made to include, in addition, mycosis fungoides, Cohnheim's pseudo-leukemia<sup>2</sup> and related conditions. The facts on which this opinion is based were derived largely from the microscopic study of forty-seven cases of Hodgkin's disease observed at the New York and Bellevue Hospitals, in seven of which postmortem investigation was permitted, and partly from anatomic and histologic observations carried out in conjunction with Dr. J. Frank Fraser<sup>3</sup> in seven cases of mycosis fungoides.

The first observable change in the lymph nodes in Hodgkin's disease consists in hyperplasia of lymphoid cells, with regeneration of the germinal follicles. This observation was made independently by Longcope,<sup>4</sup> Symmers<sup>5</sup> and Cunningham,<sup>6</sup> and is susceptible of substantiation by microscopic examination of the smaller nodules commonly to be found at the periphery of the massive enlargements in the neck, axilla and elsewhere. Incidentally, this histologic feature may constitute a source of error in the determination of the nature of the process, since the change in question is identical with that to be found in similar situations in tuberculous lymphadenitis, benign lymphoma, pseudoleukemia, and other conditions which are conceived in simple lymphoid hyperplasia and in which differentiation at the outset is consequently impossible. Similarly, the first observable histologic change in the spleen in Hodgkin's disease consists in hyperplasia of the lymphoid follicles of identical appearance with that seen in other splenic lesions, notably syphilis, tuberculosis, and status lymphaticus. Again, the first observable histologic change in the liver, kidney, bone marrow and other tissues in Hodgkin's disease consists in the appearance of minute lymphomatous foci indistinguishable from those encountered in the

2. Cohnheim: *Virchows Arch. f. path. Anat.*, 1865, **33**, 451. Symmers, Douglas: *THE ARCHIVES INT. MED.*, 1909, **4**, 218. See Footnote 5.

3. To be published.

4. Longcope: *Bull. Ayer Clin. Lab.*, 1903, **1**, 4.

5. Symmers, D.: *Certain Unusual Lesions of the Lymphatic Apparatus, Including a Description of Primary Hodgkin's Disease of the Spleen and a Case of Gastro-Intestinal Pseudoleukemia*, *THE ARCHIVES INT. MED.*, 1909, **4**, 218.

6. Cunningham: *Am. Jour. Med. Sc.*, 1915, **101**, 868.



same situation in related diseases. In other words, the preliminary hyperplasia of lymphoid cells in Hodgkin's disease is not to be distinguished microscopically from that which occurs in the same locality in a half dozen other conditions, and it is only when histologic developments have trespassed beyond this point that differentiation becomes possible. As development proceeds, however, the hyperplastic lymph nodes in Hodgkin's disease begin to lose their architectural identity, the germinal follicles disappear, the lymph cords are merged into broad sheets of proliferating lymphoid cells, and at least two alien cells make their appearance and lend individuality to the microscopic picture. One of these cells is to be found in varying numbers lying free in the lymph sinuses. It is a large, rounded cell, indistinguishable from that frequently to be observed in other tissues, but bearing a striking resemblance to the nongranular, mononuclear cells of the normal bone marrow. The other is a much larger cell of identical morphology with that encountered in the normal bone marrow in the form of myeloplaxes. In the lymph nodes in the early stage of Hodgkin's disease these myeloid giant cells lie free among the lymphoid cells, and, in a later stage, may be seen as individual cells resting in the tissue spaces of a lymph node which has now become more or less richly permeated by delicate fibrous trabeculae. In the liver, spleen and other tissues affected by Hodgkin's disease the histologic changes are identical with those in the lymph nodes, or, at all events, there is no essential difference, and the microscopic diagnosis may be made with equal facility.

There is still another cell which forms an important feature of the pathologic histology of Hodgkin's disease, and which is found in varying numbers in a large percentage of all cases, namely, the polynuclear eosinophil. The significance of this cell in Hodgkin's disease, as elsewhere, is not altogether clear. There is an apparently closely related cell whose presence as part of the histologic change in Hodgkin's disease seems to have been overlooked, or whose identity has been confused with that of the eosinophil, namely, the eosinophilic myelocyte. It occurs in varying numbers in the lymph nodes in about a third of all cases. Sometimes it is the only eosinophilic cell present; at other times it is associated with the presence of polynuclear eosinophils, from which it is to be differentiated by the fact that it possesses but a single nucleus. Otherwise its appearance in the fixed tissues is identical. In a case of Hodgkin's disease which recently came under my notice, the lymph nodes presented vast hordes of eosinophilic myelocytes arranged in clumps of such size as to occupy a large part of the low power field of the microscope, and, in a second case, myelocytes were present in excessive numbers in the lymph nodes and visceral nodules, and in the blood as part of a terminal leukocytosis.



The detection of eosinophilic myelocytes in the lymph nodes is a further indication that Hodgkin's disease is associated with disturbances in the bone marrow, and their presence in that situation is reasonably to be ascribed to the fact that the marrow has not been able to supply eosinophils in adequate numbers, and has consequently been forced to disgorge a similarly granulated substitute.

In various forms of helminthiasis there is an eosinophilia in the blood varying between 5 per cent. and 85 per cent., and at the site of attachment of ankylostomata to the intestinal mucosa it is said that there is often a marked accumulation of eosinophils (Todd). It is assumed, therefore, that certain parasitic worms manufacture and inject into their host substances which are not only capable of stimulating the expulsion of eosinophils from the bone marrow, but which are also capable of attracting eosinophils toward the source of the stimulus. A similar explanation appears to be applicable to the appendix in certain subacute infective lesions attended by enormous infiltration of eosinophils. The fallopian tubes also offer a familiar example of localized infiltration of eosinophils. In this locality the process seems to bear a relationship to the subsiding stage of gonorrheal infection, and doubtless the chemotactic attraction is to be sought in the autolyzing pus so frequently associated with this variety of fallopian salpingitis.

The histologic alterations in the lymph nodes in Hodgkin's disease are aptly comparable to the so-called myeloid transformation of the hemopoietic viscera, notably the spleen and liver, in primary progressive pernicious anemia, and in certain sclerotic and hyperplastic lesions of the bone marrow. Thus, of seventeen cases of pernicious anemia, Gulland and Goodall<sup>7</sup> found myeloid foci in the liver in eight, and myelocytic infiltration of the spleen in four, while in still another case the spleen exhibited typical bone marrow giant cells. Donhauser<sup>8</sup> has recorded an example of splenomegaly associated with hyperplastic changes in the bone marrow in which the spleen was the seat of innumerable myeloid foci composed of large mononuclear, nongranular cells, giant cells, myelocytes and normoblasts, the histologic picture, with the nucleated red cells excluded, presenting such a striking resemblance to that of the spleen in Hodgkin's disease as to render differentiation a matter of extreme difficulty. In this connection it is interesting to note that the anemia of Hodgkin's disease seldom approaches the pernicious type, so that the bone marrow does not undergo megaloblastic changes, but retains its ability to produce mature cells in sufficient numbers to meet the customary demands, thus obviating the necessity for auxiliary foci of red cell hematogenesis.

7. Gulland and Goodall: *Jour. Path. and Bacteriol.*, 1905, **10**, 125.

8. Donhauser: *Bull. Ayer Clin. Lab.*, 1908, **5**, 46.

In Hodgkin's disease the bone marrow often presents profound structural alterations. In one group of cases there is overgrowth of connective tissue resulting in obliteration of the marrow cavity. This is apt to be limited to certain parts of the marrow system or even to a focal distribution in individual bones. In a second group, the bone marrow is the seat of histologic changes of essentially the same character as those encountered in the lymph nodes and elsewhere. Finally, there is a third group of cases attended by extraordinary hyperplastic changes in the cells of the bone marrow, the increase affecting principally the myelocytes and the large nongranular cells of the lymphocytic type. Normoblasts may be present in increased numbers, but megaloblasts have never been described in any of the reports with which I am familiar, nor have I ever found them in any of the preparations that I have examined. The bone marrow giant cells are not increased, and may even be conspicuous by their diminished numbers — a fact which finds ready explanation in the occurrence of myeloid giant cells in such large numbers in the tissues beyond the osseous system as to impoverish the supply in the marrow, where, at best, giant cells are by no means numerous.

In short, the changes in the marrow in Hodgkin's disease are such as to provide a perfectly clear and logical explanation for the alterations in the extramedullary hemopoietic system and in such other viscera as may be involved. Whether the changes in the marrow are primary or not has no essential bearing. The fact remains that the presence of myeloid giant cells, eosinophils and eosinophilic myelocytes in the lymph nodes and elsewhere is an indication of disturbances in the bone marrow resulting in increased expulsion of these cells and their deposition in remote tissues. The only other possible explanation is that the myeloid changes in the hemapoietic viscera are reversionary — that the hemopoietic function of the lymph nodes, liver and spleen has been re-awakened, the occurrence of myeloid foci in them representing the structural expression of their rejuvenation. This explanation is, however, inapplicable to the organs beyond the hemopoietic system, namely, the kidney, suprarenal, lung, thyroid, etc., in all of which Hodgkin's disease sometimes initiates histologic changes similar to those in the lymph nodes and elsewhere. It is true that the human fetal spleen normally contains cells of the bone marrow type in the form of giant cells and myelocytes, all of which diminish and disappear as development proceeds. In the spleen of certain other mammals, however, myeloid foci persist throughout life. It is quite possible, therefore, that the human spleen may revert to the hematogenetic function of embryonal life, and the same is true of the liver; but as far as the lymph nodes are concerned, the explanation is



decidedly strained, since the embryonal lymph node is not an auxiliary of the marrow system, functionally or otherwise, and certainly the fully developed lymph node is not apt to assume a duty so remote from its own as to include the manufacture of alien cells. The conclusion, it seems to me, is inevitable, that the presence of myeloid giant cells and eosinophilic myelocytes in the lymph nodes and elsewhere in Hodgkin's disease is made possible only by the process of embolism from the bone marrow. Moreover, this explanation is strictly comparable to the discharge of normoblasts into the circulation after severe hemorrhage, to the occasional presence of myeloid giant cells in the capillaries of the liver and lungs, and to the enormous influx of giant cells and myelocytes into the spleen of certain lower animals following infection by the bacillus of hog cholera, pyocyaneus, anthrax and other micro-organisms, and after repeated artificial hemorrhages.<sup>9</sup> In short, the interpretation in question is supported by both precedent and principle.

In a certain number of all cases of Hodgkin's disease necroses are present in the affected tissues, and the irregular type of temperature so frequently to be observed in the living patient is probably due to absorption of the products of necrosis, plus those derived from the more deliberate disintegration of cells. Whether the same explanation applies to the chronic relapsing fever described by Murchison,<sup>10</sup> Pel,<sup>11</sup> Ebstein<sup>12</sup> and others, is yet to be seen. The fact remains, however, that this type of fever is always associated with the presence of enormous numbers of focal necroses in the tissues involved, particularly the spleen. This explanation of the temperature disturbances in Hodgkin's disease is analogous to that usually offered for the fever so commonly observed in patients suffering from hypernephroma, in which variety of neoplasm necrotic and degenerative changes are almost constant. The contention likewise finds confirmation in the negative evidence offered by the lymphoid tissues in lymphosarcomatosis, where necroses are uncommon and disturbances of temperature are correspondingly infrequent.

Finally, the theory above outlined does not attempt to explain why a particular group of lymph nodes should become affected in Hodgkin's disease and apparently remain alone involved for months or years. The same general problem arises in connection with the occurrence and distribution of metastases in malignant growths, and seems, at the present moment, to be beyond the field of the explicable, unless, of course, it is regarded as another expression of chemotaxis. For

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9. Pugliese: *Fortschr. d. Med.*, 1897, **15**, 727. Opie: *Am. Jour. Med. Sc.*, 1904, **127**, 988.

10. Murchison: *Tr. Path. Soc.*, London, 1871, **21**, 372.

11. Pel: *Berl. klin. Wchnschr.*, 1887, **24**, 644.

12. Ebstein: *Berl. klin. Wchnschr.*, 1885, **22**, 565 and 837.



example, there is no apparent reason why certain tumors of the thyroid, prostate, breast, adrenal, etc., should give rise to secondary deposits in the bone marrow with such appalling freedom, nor, conversely, is it possible satisfactorily to explain the relative immunity of the spleen to metastatic growth, nor the almost complete immunity of the prostate to syphilis, and of the pancreas to tuberculosis. It is nevertheless true that, in Hodgkin's disease, a group of lymph nodes may become enlarged and remain for an almost indefinite period as the sole objective manifestation of a systemic process.

#### SUMMARY

Hodgkin's disease is primarily neither an infective nor a neoplastic lesion of the lymph nodes, but a systemic disease which expresses a predilection for lymphoid tissues, giving rise to multiple foci of growth at approximately the same time and in response to the same provocative agent. The provocative agent, whatever its nature and origin may be, causes preliminary hyperplastic changes in the lymphoid tissues and initiates disturbances in the bone marrow, characterized, among other things, by proliferation of the nongranular mononuclear cells of the lymphocytic type, eosinophils and eosinophilic myelocytes. These cells, together with the myeloplaxes, are thrown into the circulation and filtered out by the lymph nodes or deposited in them in response to chemotactic attractions, the fibrotic changes in the recipient tissue representing a purely local reactive process. The histologic changes beyond the lymphoid system proper, namely, in the liver, kidneys, etc., represent a reaction on the part of normally existing lymphomatous foci to the same toxic substance which is responsible for the disturbances in the bone marrow and for the myeloid transformation of the lymph nodes.

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# THE TREATMENT OF SYPHILIS OF THE CENTRAL NERVOUS SYSTEM

## A COMPARISON OF MERCURIALIZED SERUM AND SALVARSANIZED SERUM \*

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Cases of syphilis with positive findings in the spinal fluid treated during a period of three years following the opening of the Peter Bent Brigham Hospital may be divided into three groups. The patients of one group were treated with salvarsan, mercury and potassium iodid for a sufficiently long time to determine whether or not clinical and laboratory improvement would follow. If there was definite improvement, no change was made in the therapy. If improvement did not follow after a reasonable length of time, they were shifted into a second group the members of which received in addition to the above, salvarsanized serum intraspinally. In a third group were placed those patients having negative blood Wassermann reactions, and to them salvarsanized serum alone was given. Seventy-five patients were treated under this plan, and to them 450 intraspinal injections of salvarsanized serum were given in addition to the intravenous salvarsan and other medication. A detailed report of this work has appeared in a recent publication.<sup>1</sup>

At the end of this time, having satisfied ourselves concerning the relative efficiency of these different modes of treatment and the results to be derived from each, we decided to discontinue the use of salvarsanized serum and to substitute for it mercurialized serum prepared by the method suggested by Byrnes.<sup>2</sup> The very obvious advantages of the mercurialized serum are, first, no previous treatment is necessary in its preparation, so where salvarsan is not needed, inconvenience and expense are avoided; second, the serum can be obtained at any time convenient for the patient and the physician rather than on the exact minute, as is the case with the salvarsanized serum; third, a large supply can be made up at one time and the individual

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\* From the Medical Clinic of the Peter Bent Brigham Hospital.

1. Walker, I. C., and Haller, D. A.: The Treatment of Syphilis of the Central Nervous System with Intravenous Salvarsan Alone, with Intravenous Salvarsan and Intraspinal Salvarsanized Serum Together, and with Intraspinal Salvarsanized Serum Alone, *THE ARCHIVES INT. MED.*, 1916, **18**, 376.

2. Byrnes, C. M.: *Jour. Am. Med. Assn.*, 1914, **63**, 2182.

doses can be used as needed over a period of several weeks, thus saving an enormous amount of labor and time. We were further influenced in trying this method ourselves, as we believed that a comparison of cases treated by the same workers using different methods would be of more value than a comparison of results obtained by different men, because of the uniformity of technic used in the various tests, particularly the Wassermann reactions, and because of a constant personal equation in judging results obtained. It was possible also to use the mercurialized serum in the treatment of patients who had already received several doses of salvarsanized serum, and in this way form an opinion concerning the degree of reaction caused by the two serums in the same group of patients.

The present paper consists of a description of the preparation of mercurialized serum as used in the cases here reported, and of a tabulation of the results obtained from its use in forty-five patients to whom 150 doses were given, with a comparison of these results and those obtained in previous cases with salvarsanized serum. To facilitate comparison, the cases reported here will be numbered in sequence with those previously reported.

*Technic.*—In the preparation of mercurialized serum, blood is withdrawn from a vein and allowed to clot. The expressed serum is centrifuged in order to free it from cells. The serum is then pipetted into glass tubes in 8 c.c. amounts, and to each tube is added 0.001 gm. of mercuric chlorid in a 0.1 per cent. aqueous solution. The fluid is agitated for a few moments in order to insure a thorough mixing. The white ring of precipitated albuminate of mercury quickly redissolves in the excess of serum, giving a perfectly clear solution. The tubes are plugged and the serum inactivated for thirty minutes at 56 C. They are then stored away on ice until needed, when they are warmed to body temperature and the serum is administered.

The amount of serum used has seemed to be of relatively little importance, although excessive amounts probably add to the immediate reaction. From 6 to 8 c.c. has been the amount used in most of the doses given in this series, for the reason that about that amount of spinal fluid is usually withdrawn for cell count, globulin and Wassermann tests. The dose of mercuric chlorid has been varied from 0.0005 to 0.002 gm. The degree of immediate reaction is not perceptibly changed by increasing the amount to 0.001 gm., but an increase above this figure has seemed to add materially to the discomfort of the patient. The length of time during which the serum was heated to 56 C. has been varied from twenty minutes to two hours without apparently affecting the degree of reaction. The age of the serum does not perceptibly add to its irritating properties. Several doses kept on ice for eight weeks and more were followed by less disturbance than many which were administered within twenty-four hours after preparation.

The reactions in a given patient following the intraspinal administration of irritating drugs, whether salvarsanized serum or serum to which either mercury or salvarsan has been added, are of the same type and vary only in degree. Headache, slight fever, nausea and general malaise are to be expected, and these symptoms do occur in a moderate number of cases. Severe lightning pains follow treatment



in many cases. They are more apt to occur in patients who have previously been troubled with them; many patients who have had no pains for months or even years, often have them recur after treatment. This immediate reaction ordinarily passes off within twelve to twenty-four hours. In about 10 per cent. of individuals it will last longer, and occasionally, but not often, will necessitate two or three days' stay in the hospital after treatment, and several doses of morphin. In our series of cases severe reactions of this kind have been more than three times as frequent after mercurialized serum than after salvarsanized serum. Two patients have, on the day following treatment with mercurialized serum, developed a stiff neck and positive Kernig sign, with a temperature reaching 102 F. This condition did not persist in either case longer than twenty-four hours and there were no sequelae. Subsequent treatments given to these patients have caused no great disturbance. Occasionally intraspinal treatment with either salvarsanized serum or mercurialized serum will precipitate a gastric crisis in a tabetic patient subject to these attacks. In one instance an attack thus begun lasted for a week. Two patients, on the other hand, who were treated during attacks, have had the attack promptly terminate. This has been repeated on several subsequent occasions with one of these patients. The other has never had a recurrence. Temporary increase in tabetic symptoms, ataxia, difficulty in urination, incontinence and paresthesias may occur in the first two or three days following treatment with either serum, and rarely these symptoms last for a week or two. Only one patient has been greatly troubled, and this trouble was lessened by lengthening the interval between treatments. A patient whose case was diagnosed as general paresis, who had had a complete hemiplegia ten years before, with partial recovery, suffered a sudden and complete paralysis of the same side again following his fifth intraspinal treatment with salvarsanized serum. This confined him to bed for seven weeks. At the present time he walks better and uses his hand and arm as well, if not better, than he did before any treatment. This has been our most alarming experience in a total of 600 intraspinal treatments. It seems fair to say that the immediate reaction following either salvarsanized serum or mercurialized serum is one which can be regulated by dosage. We believe that the reaction following 0.001 of mercuric chlorid in 8 c.c. of normal serum is, as a rule, much more severe than that caused by 20 c.c. of undiluted salvarsanized serum. The unusual complications which occur may follow either, but it is reasonable to assume that they are more apt to occur where the irritation is greatest. In the administration of 150 doses of mercurialized serum we have had about as many of them occur as in 450 treatments with salvarsanized serum. Eleven patients have been treated with at least three doses of each kind of serum. In every case

the reaction has been noticeably more severe following the administration of mercurialized serum.

The dosage and frequency of treatment with mercurialized serum depend altogether on the individual features of each case, just as they do with salvarsanized serum. Certain fairly definite rules can, however, be followed. The first dose with every case should be half strength. Later, when the spinal fluid Wassermann reaction is positive only with large amounts of fluid, treatments should be given at greater intervals. Cases of general paresis, active meningitis and cerebrospinal syphilis stand treatment much better than cases of tabes do. In this group of cases the dose each time can be a maximum one and a minimum interval of time between treatments can be safely

TABLE 1.—TO SHOW THE IMMEDIATE EFFECT OF AN IRRITANT ON THE SPINAL FLUID CELL COUNT

Case No.	Date of 1st Lumbar Puncture	Cells per C.mm.	Date of First Treatment	Cells per C.mm.	Date of Second Treatment	Cells per C.mm.	Date of Third Treatment	Cells per C.mm.
Mercurialized Serum								
94	4/14/16	142	5/22/16	140	5/26/16	300*	.....	...
91	6/17/16	34	6/21/16	30	6/27/16	62	.....	...
81	9/ 2/16	156	9/10/16	145	9/16/16	410*	.....	...
79	.....	...	9/ 5/16	291	9/ 9/16	187	9/11/16	300*
82	.....	...	9/ 4/16	175	9/ 8/16	100	9/12/16	180
100	9/15/16	8	9/18/16	8	10/ 2/16	74	.....	...
101	9/27/16	56	9/29/16	50	10/ 4/16	84	.....	...
Salvarsanized Serum								
27	10/28/14	100	11/ 4/14	37	11/11/14	165	.....	...
24	8/21/14	3	1/27/15	3	2/ 2/15	87	.....	...
64	10/11/15	37	10/17/15	35	10/28/15	64	.....	...
62	.....	...	1/ 7/15	11	1/12/15	123	1/19/15	216*

\* Few red cells also present.

allowed. Several patients with meningitis and cerebrospinal syphilis have been given two treatments intraspinally each week for two or three weeks without severe reactions in any case. Two patients with tabes now under treatment cannot stand the administration of intraspinal serum more often than once in three months.

The immediate effect of either serum on the spinal fluid cell count often is to cause an increase in cells. The cell count then falls during the next few days to a point below the previous count. Occasionally, after a severe reaction, the count goes up considerably and red cells appear in the fluid. This is found much more commonly after the use of mercurialized serum than after salvarsanized serum (see Table 1).



A comparison of the efficacy of the two serums in relieving symptoms and in causing objective changes in signs and in the laboratory findings, offers more difficulties than are encountered in a comparison of the reactions from treatment, for while the individual susceptibility to reaction from intraspinal treatment varies, the amount of treatment needed to accomplish the same result in two different cases with the same diagnosis varies to a still greater degree. Evidently, then, the only fair comparison is a comparison of the general averages in fairly large groups of cases. We have attempted this in several ways which can be best understood by a study of the accompanying tables.

In Table 2 there are thirty patients who received a total of 104 doses of salvarsanized serum in addition to intravenous salvarsan. By subtracting the last cell count from the first listed in each case the total drop in cells for that case is found. The total fall for the 30 cases divided by the total number of doses given shows the fall in cells per dose. This fall in the group treated with salvarsanized serum averaged 14 per treatment. The 15 patients listed in Table 3 received a total of 55 doses of mercurialized serum, and here the average drop in cells per treatment calculated in the same way was 27, or twice that obtained in those patients treated with salvarsanized serum.

In the same way by subtracting the amount of spinal fluid necessary for complete fixation before any treatment, from that necessary after three or four doses of salvarsan and intraspinal serum, the change in the Wassermann reaction in cubic centimeters induced by treatment is found. In the cases of Table 2 this averaged 0.04 c.c. per treatment. The average in the second group treated with mercurialized serum was 0.08 c.c., or again twice as great an average change as that induced by salvarsanized serum. In the last column of this table remarks are found which bear on the clinical changes. The comparison here is somewhat in favor of the salvarsanized serum, as all the patients in Table 2 showed indications of improvement, or at least of remission of symptoms. Two patients treated with salvarsan and mercurialized serum showed no change. One of these was a case of general paralysis, the other was a case of *tabes dorsalis* with gastric crises.

Twenty-seven patients have been treated by the intraspinal route alone. Of these, seventeen were given salvarsanized serum and ten mercurialized serum. The findings in the spinal fluid with the changes resulting from the first three or four treatments are tabulated in Table 4.

The average drop in cell count per treatment in those patients given salvarsanized serum was twenty-one, while the average in those given mercurialized serum was twenty-six, or practically the same figure (Tables 4 and 5). The average change in the strength of the Wasser-



TABLE 2.—To SHOW THE EFFECT OF THREE OR FOUR DOSES OF INTRAVENOUS SALVARSAN AND INTRASPINAL SALVARSANIZED SERUM ON THE CELL COUNT, THE WASSERMANN REACTION, AND THE CLINICAL SYMPTOMS

Case No.	Cell Count	Salvar., Gm.	Serum, C.c.	Cell Count	Salvar., Gm.	Serum, C.c.	Cell Count	Salvar., Gm.	Serum, C.c.	Cell Count	Salvar., Gm.	Serum, C.c.	Change in Wassermann Reaction in C.c.	Remarks
39	128	0.3	20	38	0.4	18	25	0.4	20	19	0.4	20	0.5 + to 0.8 + *	Pain and ataxia less
21	166	0.3	20	11	0.4	20	16	0.4	20	..	...	..	0.05 + to 0.1 +	Headache and paresthesia relieved
11	145	0.3	14	90	0.4	22	39	0.5	18	10	0.4	20	0.05 + to 0.2 +	Headache, aphasia and diplopia relieved
15	108	0.4	16	..	0.4	18	..	0.5	20	31	...	..	None	Incontinence and ataxia less
17	90	0.4	14	47	0.5	20	13	0.5	20	15	0.5	22	0.2 + to 0.3 +	Pain relieved; ataxia less
18	84	0.4	20	40	0.4	20	16	0.4	23	10	0.4	26	0.2 + to 0.3 +	mentally improved
36	86	0.4	20	63	0.5	20	40	0.6	20	30	0.6	22	0.2 + to 0.3 +	Pain relieved
29	87	0.3	18	86	0.6	18	15	0.4	25	11	...	..	0.1 + to 0.2 +	Ataxia and paresthesia relieved
1	35	0.4	18	7	0.5	20	2	0.6	16	1	0.6	20	None	Incontinence relieved
6	32	0.3	20	30	0.4	20	20	0.5	18	10	...	..	0.2 + to 0.3 +	Pain relieved
45	14	0.6	16	5	0.6	18	4	0.5	25	..	...	..	1.0 + to 1.0—	Paresthesia relieved
48	37	0.2	18	16	0.4	20	25	0.6	18	8	0.6	20	0.2 + to 0.5 +	All symptoms relieved
44	33	0.5	20	21	0.5	22	30	0.4	20	20	0.5	20	None	All symptoms except ataxia relieved
41	45	0.3	20	17	0.6	25	..	0.6	26	7	...	18	0.3 + to 0.6 +	Tremor relieved; mentally improved
43	40	0.3	18	33	0.4	22	15	0.4	26	10	0.4	22	0.1 + to 0.2 +	Gastric symptoms relieved
13	43	0.5	20	13	0.6	20	10	0.6	10	5	...	..	0.05 + to 0.2 +	Pain, headache and dizziness relieved
20	57	0.6	18	16	0.5	20	16	0.5	22	7	...	..	0.3 + to 0.4 +	Pain and vomiting relieved
10	47	0.6	16	55	0.6	18	28	0.5	20	16	0.5	20	0.2 + to 0.4 +	Ataxia relieved
14	35	0.4	20	26	0.5	20	20	0.5	20	16	0.5	25	0.1 + to 0.2 +	Pain and vomiting relieved
19	18	0.4	16	9	0.5	18	5	0.4	20	5	0.4	18	None	Pain and dizziness relieved
8	63	0.4	18	34	0.5	20	9	0.6	18	5	...	..	0.3 + to 0.4 +	Headache and dizziness relieved
9	25	0.4	20	20	0.5	22	8	0.5	20	7	...	..	0.3 + to 0.4 +	Pain relieved
5	26	0.4	20	9	0.6	20	10	0.6	18	9	...	..	0.2 + to 0.3 +	Pain relieved
16	16	0.3	18	10	0.5	20	3	0.4	16	3	0.6	23	None	Pain and dizziness relieved
65	36	0.3	20	29	0.5	25	6	0.6	20	4	0.4	20	0.1 + to 0.8 +	Diplopia relieved; mentally improved
47	21	0.3	20	7	0.4	25	3	0.4	26	1	0.4	20	0.2 + to 0.3 +	Pain relieved
40	18	0.3	18	11	0.5	20	16	0.4	28	15	0.4	..	0.2 + to 0.5 +	Ataxia lessened
64	64	0.4	23	30	0.4	20	9	...	20	..	...	..	0.5 + to 0.6 +	Pain relieved
51	39	...	16	32	0.3	21	20	0.4	22	9	...	..	0.4 + to 0.6 +	Pain relieved
74	16	0.6	18	5	0.6	15	2	...	..	..	...	..	0.2 + to 0.4 +	Pain relieved

\* Throughout this paper + means "complete fixation."

TABLE 3.—To SHOW THE EFFECT OF THREE OR FOUR DOSES OF INTRAVENOUS SALVARSAN AND INTRASPINAL MERCURIALIZED SERUM ON THE CELL COUNT, THE WASSERMANN REACTION, AND THE CLINICAL SYMPTOMS

Case No.	Cell Count	Salvar., Gm.	Mer- cury, Gm.	Cell Count	Salvar., Gm.	Mer- cury, Gm.	Cell Count	Salvar., Gm.	Mer- cury, Gm.	Cell Count	Change in Wassermann Reaction In C.e.	Remarks
75	15	0.3	0.001	27	0.4	0.001	7	0.4	0.001	4	None	All symptoms relieved
76	60	0.4	0.001	32	0.9 neo	0.001	10	0.9 neo	0.001	7	0.1+ to 0.2+	Pain relieved; ataxia lessened
77	60	0.3	0.001	25	0.4	0.001	6	0.4	0.001	16	0.2+ to 0.8+	Pain and dizziness relieved
78	520	0.3	0.001	201	0.6	0.001	107	0.6	0.001	15	0.6+ to 0.8+	All symptoms relieved
79	201	0.4	0.001	187	0.6	0.001	300	0.6	0.001	74	0.2+ to 1.0+	Aphasia gone; vomiting stopped
80	44	0.3	0.001	15	0.4	0.001	2	0.4	0.001	..	0.1+ to 0.2+	Less pain
81	156	0.4	0.001	410	0.4	0.001	142	0.4	0.001	42	None	Headaches relieved
82	175	0.6	0.001	100	0.6	0.001	118	0.6	0.001	45	None	Great mental improvement
83	52	0.4	0.001	30	0.6	0.001	11	0.6	0.001	..	0.2+ to 0.6+	Less pain; no vomiting
84	66	0.4	0.001	40	0.4	0.001	41	0.4	0.001	20	0.1+ to 0.5+	Headache and vomiting gone
85	36	0.4	0.001	36	0.6	0.001	27	0.6	0.001	..	0.2+ to 0.3+	No change
86	71	0.4	0.001	31	0.6	0.001	21	0.6	0.001	..	0.2+ to 0.4+	Pain relieved
87	11	0.6	0.0005	10	0.6	0.001	5	0.6	0.001	..	0.4+ to 0.5+	Pain lessened
88	136	0.3	0.001	130	0.6	0.001	10	0.4	0.001	..	0.3+ to 0.5+	Headache and gastric symptoms relieved
101	56	0.4	0.001	20	0.6	0.001	84	0.4	0.001	..	0.2+ to 0.6+	No change

TABLE 4.—TO SHOW THE EFFECT OF THREE OR FOUR DOSES OF INTRASPINAL SALVARSANIZED SERUM ALONE ON THE CELL COUNT, THE WASSERMANN REACTION AND THE CLINICAL SYMPTOMS

Case No.	Cell Count	Salvar. Serum, C.c.	Cell Count	Salvar. Serum, C.c.	Cell Count	Salvar. Serum, C.c.	Cell Count	Change in Wassermann Reaction in C.c.	Remarks
25	40	15	30	25	14	27	5	None	Pains relieved
27	210	14	100	27	37	20	165	0.4+ to 0.5+	Pains relieved; ataxia less
34	52	16	26	20	30	18	5	0.1+ to 0.2+	Pain and dizziness relieved
25	20	15	3	30	3	25	0	None	Pain and blizziness relieved
4	15	20	10	20	9	20	4	0.4+ to 0.5+	Pain and headache relieved
38	103	20	48	20	40	18	27	0.2+ to 0.3+	Pain relieved
22	7	20	6	15	6	20	6	0.5+ to 0.6+	Pain relieved; ataxia less
24	7	14	5	30	3	21	3	0.4+ to 0.6+	Temporary relief from gastric crises
33	5	15	3	20	3	20	1	0.6+ to 0.9+	Pain and numbness relieved
32	60	20	23	18	11	20	..	1.0 negative	Pain and headache relieved
31	27	15	13	20	7	25	..	1.0 negative	Pain relieved and ataxia lessened
12	43	15	20	20	9	20	..	0.3+ to 0.4+	Headache relieved
35	150	20	75	20	20	20	12	None	Headache and diplopia relieved
23	533	20	272	20	100	25	46	0.1+ to 0.2+	All symptoms relieved
64	30	16	9	20	15	25	3	0.5+ to 0.8+	Pain relieved
66	29	15	16	15	12	20	10	None	Pain relieved
50	16	16	11	20	11	20	5	0.6+ to 0.7+	Pain relieved



TABLE 5.—To SHOW THE EFFECT OF THREE OR FOUR DOSES OF INTRASPINAL MERCURIALIZED SERUM ALONE ON THE CELL COUNT, THE WASSERMANN REACTION AND THE CLINICAL SYMPTOMS

Case No.	Cell Count	Mercury, Gm.	Cell Count	Mercury, Gm.	Cell Count	Mercury, Gm.	Cell Count	Change in Wassermann Reaction in C.c.	Remarks
90	107	0.001	58	0.001	22	0.001	16	0.2+ to 1.0 Neg.	Gain in weight; less ataxia
91	34	0.001	20	0.001	62	0.001	8	No change	No change
92	27	0.001	18	0.001	3	0.001	4	0.4+ to 0.8+	Relief of all pain
93	70	0.001	25	0.001	15	0.001	7	0.3+ to 0.6+	No change
94	142	0.001	300	0.001	36	0.001	20	0.2+ to 0.5+	Relief of pain and dizziness
95	100	0.001	30	0.001	12	0.001	7	0.5+ to 1.0+	Pain relieved
96	44	0.001	0	0.001	2	.....	..	No change	All symptoms relieved
97	6	0.001	6	0.001	2	0.001	3	0.3+ to 1.0 Neg.	All symptoms lessened
98	200	0.001	43	0.001	..	.....	..	No change	Pain lessened
99	20	0.001	6	0.001	..	.....	..	No change	Pain relieved

mann reaction per treatment interpreted as before in cubic centimeters of spinal fluid necessary for complete fixation was 0.03 in those treated with salvarsanized serum. In those treated with mercurialized serum the average was 0.13, or more than four times as great a change. From these figures it appears that mercurialized serum in this dose is a more efficient antisyphilitic than salvarsanized serum as measured by a drop in the strength of the Wassermann reaction. It appears, however, from the preceding comparison of the reactions produced by the two serums that mercurialized serum is so much more irritating that it cannot be used with the frequency with which salvarsanized serum can, and this is especially true of its use in *tabes dorsalis*. Consequently, the results at the end of a year of treatment, if each serum were used to the greatest extent consistent with safety, probably would not show such a discrepancy against salvarsanized serum because of the larger number of doses which could be given.

A comparison of the ultimate results obtained with the individual cases in the two groups is impracticable because of the differences in the total amount of treatment which has been given to the two groups, and also because of the small interval of time which has elapsed since treatment was discontinued, or because many of the group treated with mercurialized serum are still under treatment. The condition of the individual cases can best be described individually, and this is done in the case reports.

#### CONCLUSIONS

1. The irritating effect in the spinal canal of serum to which mercuric chlorid has been added in the dose of 0.001 gm. is greater than that of 20 c.c. of salvarsanized serum separated from blood drawn thirty minutes after a dose of 0.6 gm. of salvarsan.
2. The average effect on the laboratory findings in the spinal fluid from one dose of mercurialized serum is greater than from one dose of salvarsanized serum.
3. Unpleasant symptoms are more common following intraspinal mercurialized serum than following salvarsanized serum.
4. The greater irritation of the meninges from mercurialized serum prevents as rapid repetition of dosage as is possible with salvarsanized serum.
5. Cases of general paresis, meningitis and cerebrospinal syphilis stand intraspinal treatment with mercurialized serum better than do cases of *tabes dorsalis*. It is particularly in cases of active syphilis of the meninges that the mercurialized serum is useful.
6. Mercurialized serum has an advantage over salvarsanized serum in ease of preparation and in its keeping qualities. For these reasons

it can be used under clinical conditions in which the use of salvarsanized serum is impossible, or at least very much more difficult.

## REPORT OF CASES

CASE 75.—A man, aged 48, entered the hospital March 27, 1916. A diagnosis of *tabes dorsalis* was made. Twenty-seven years before he had had gonorrhea. He had had no treatment for syphilis and did not know of his infection. For two years he had had attacks of nausea and vomiting lasting for from eight to ten days, coming on about every month. He had had a chronic deforming arthritis for several years.

Physical examination showed unequal, irregular pupils which did not react to light. His knee jerks were present and equal. There was no Romberg sign. Both elbows were partially flexed and almost completely fixed in that position.

The Wassermann reaction in the blood serum was positive. The spinal fluid gave a positive Wassermann reaction and showed a cell count of 15 per c.mm.

He was given five doses of salvarsan and five intraspinal treatments with mercurialized serum. Two months after his first treatment he became free from gastric symptoms and has remained so up to the present time. The Wassermann reaction was unchanged in his blood and spinal fluid. The cell count in the spinal fluid became normal.

CASE 76.—A man, aged 51, entered the hospital March 25, 1916. A diagnosis of *tabes dorsalis* was made. He had had a chancre twenty years before. He had had shooting pains in his legs for eight years. For two years he had been ataxic and for one year had had incontinence of urine. For six months he had had girdle sensation. He had lost 18 pounds in weight. His condition had not been influenced by repeated intravenous injections of salvarsan, mercury inunctions and potassium iodid by mouth.

Physical examination showed Argyll Robertson pupils, absent knee and ankle jerks and a positive Romberg sign.

The Wassermann reaction was positive in both blood serum and spinal fluid, with a spinal fluid cell count of 60 per c.mm. He was given six intravenous doses of salvarsan and five intraspinal treatments with mercurialized serum. His pains became less severe after two treatments; after five they were almost absent. His girdle sensation disappeared. The ataxia was somewhat lessened. The Wassermann reaction in his blood serum was unchanged, in his spinal fluid it had changed by only 0.1 c.c. The cell count had fallen to 7 per c.mm.

CASE 77.—A man, aged 42, entered the hospital April 5, 1916. A diagnosis of cerebrospinal syphilis was made. In 1904 he had had a chancre. Eight months before entrance he had had a sudden complete paralysis of his right side, with inability to speak for two weeks, and retention of urine. The paralysis had slowly cleared up until he was able to walk about his room, but could do very little more. He had had pain in his neck and both arms, which was gradually becoming more severe.

Physical examination showed small, irregular pupils, which did not react to light. There was some muscle atrophy of the right side, with decided weakness of his right arm and leg. All reflexes on that side were increased and there was a positive ankle clonus, Babinski, Gordon and Oppenheim.

The Wassermann reaction in both blood and spinal fluid was positive. The spinal fluid also gave a positive globulin test and showed a cell count of 60 per c.mm.

He was given four doses of salvarsan intravenously, large amounts of potassium iodid and some mercury. He was also given six doses of mercurialized serum intraspinally. At the end of six months all pain had gone from his neck and arms, he could walk better, but still dragged his leg. His right arm was gradually recovering some power and he was doing part time work with the fire department.



His blood serum continued to give a positive Wassermann test. His spinal fluid showed a cell count of 5 per c.mm., and gave a positive Wassermann test with 0.8 c.c. whereas at entrance only 0.2 c.c. was required for complete fixation.

CASE 78.—A man, aged 36, entered the hospital Aug. 28, 1916. A diagnosis of syphilitic meningitis was made. He had never had any previous syphilitic lesions. For two months he had had frequent headaches. Five days before entrance he had had extreme headache, failing vision, increasing mental confusion, vomiting and stiffness of his neck.

Physical examination showed a general hyperesthesia, stiff neck, with pain on motion, bilateral Kernig's sign and increased reflexes. There was some mental confusion.

The Wassermann reaction was positive in both blood and spinal fluid. The spinal fluid showed a cell count of 520 per c.mm. and gave a positive globulin test. His temperature was 103 F., pulse 100 per minute and respirations 30. The white blood count was 8,400 per c.mm.

After three weeks of treatment there were no signs of meningitis remaining, the patient was free from all symptoms and was out of bed all day. He was given eight doses of salvarsan intravenously in addition to potassium iodid and mercury. He was also given six doses of mercurialized serum intraspinally. His blood Wassermann reaction continued to be positive. His spinal fluid showed a normal cell count and gave a negative Wassermann reaction and globulin test. Six weeks after he entered the hospital he returned to work and had been working steadily ever since.

CASE 79.—A man, aged 22, entered the hospital Sept. 4, 1916. A diagnosis of cerebrospinal syphilis was made. He had had a chancre five years before and had been given three doses of salvarsan intravenously. He had had no further symptoms until six months before entrance to the hospital when he had begun to have some stomach trouble and headache. These symptoms had partially cleared up after further intravenous salvarsan. One day before entrance he had suddenly lost his faculty of speech and ability to swallow and the right side of his face had become paralyzed. He had had almost constant vomiting and hiccough since that time.

Physical examination showed unequal pupils, which reacted poorly to light, a right facial paralysis and a partial paralysis of his tongue and the muscles of deglutition. There was a double Kernig's sign but no stiffness of the neck. Fundus examination showed considerable edema of both disks. There was complete aphonia, frequent vomiting and continual hiccough.

The Wassermann reaction was positive in both blood and spinal fluid. The spinal fluid showed a cell count of 300 per c.mm. and gave a positive globulin test.

Treatment consisted of mercury intramuscularly, large doses of potassium iodid by mouth, seven doses of salvarsan intravenously and seven intraspinal treatments with mercurialized serum. Two months after the first treatment his blood serum gave a weakly positive test. His spinal fluid gave a positive Wassermann reaction with 1.5 c.c., whereas at first only 0.2 c.c. was required for complete fixation. The cell count had fallen from 300 per c.mm. to 20. He showed no signs of his trouble except a slight drooping of the right angle of his mouth.

CASE 80.—A woman, aged 36, entered the hospital June 12, 1916. A diagnosis of general paresis of the insane was made. She had been married nineteen years and had one child 18 years old. She had had four miscarriages. Her husband had had general paralysis of the insane for more than two years and had been recently committed to a home for the insane. She had had pains in her legs for three years, attacks of dizziness for one year and had had numbness and paresthesia of her legs for six months.

Physical examination showed unequal and irregular pupils, which did not react to light. The knee and ankle jerks were equal but exaggerated. She

showed very definite mental instability and cried or laughed on the slightest provocation. There was some memory defect.

The Wassermann reaction was positive in both blood serum and spinal fluid. The spinal fluid showed a cell count of 44 per c.mm. and gave a positive globulin test.

She was given four doses of mercurialized serum intraspinally and six doses of salvarsan intravenously without change in her symptoms other than relief of pain.

The Wassermann reaction remained unchanged in either blood or spinal fluid. The spinal fluid cell count fell from 44 to 2 per c.mm.

CASE 81.—A woman, aged 22, entered the hospital Sept. 2, 1916. A diagnosis of cerebrospinal syphilis was made. She had had poor health all of her life. At the age of 5 she had had a rash on her face diagnosed as syphilis. One year later she had had syphilitic periostitis of both tibiae and two years later she had had syphilitic iritis of the right eye. Six months before entrance she had had general malaise, drowsiness, apathy, anorexia, numbness of her hands, headache, vertigo, polyuria, polydypsia and had lost 15 pounds of weight.

Physical examination showed the scars of an old iritis on the right, with ability to count fingers at 1 foot. Vision in the left eye was normal and the right pupil was regular and reacted to light. The tibiae showed marked changes due to syphilitic osteitis and periostitis. The urine was always of light color, with specific gravity of 1.005 and lower. The twenty-four-hour amount was usually about 2,000 c.c. The Wassermann reaction was positive in both blood and spinal fluid. The spinal fluid gave a positive globulin test and showed a cell count of 148 per c.mm.

Treatment consisted of five doses of mercurialized serum intraspinally and seven doses of salvarsan intravenously in addition to mercury intramuscularly and large doses of potassium iodid by mouth. After three weeks of treatment all headaches, dizziness, drowsiness and anorexia disappeared. She felt well and began to gain weight. Later the numbness of her hands disappeared. Treatment had no effect on her urinary symptoms.

The Wassermann reaction remained unchanged in both blood and spinal fluid. The spinal fluid cell count had fallen from 148 per c.mm. to 42 per c.mm.

CASE 82.—A man, aged 32, entered the hospital Jan. 13, 1916. Nine months before entrance he had had a chancre which was followed by a rash and sore throat, and at that time his blood Wassermann reaction had become positive. Two months after infection he had developed headache and dizziness. Three doses of neosalvarsan and fifty doses of intramuscular mercury had to some extent relieved his symptoms, but they had lately recurred and had progressed rapidly. At the time of admission he showed that he was below par mentally, with a marked memory defect. His replies to questions were vague and indefinite.

Physical examination showed unequal pupils which reacted to light. Fundus examination showed a one diopter choked disk on each side, with overfilled and tortuous veins. The deep reflexes were unchanged.

The Wassermann reaction in the blood serum was positive.

A right subtemporal decompression was performed and 10 c.c. of salvarsanized serum were injected into the lateral ventricle. The ventricular fluid removed gave a positive Wassermann reaction with 0.05 c.c., a positive globulin test, and showed a cell count of 230 per c.mm. Three days later the spinal fluid showed 240 cells per c.mm. and gave a positive Wassermann reaction with 0.05 c.c. He was given three doses of salvarsan at weekly intervals and sent home for further treatment there. Six months later he was again admitted to the hospital. At this time he was dull and stupid, disoriented for time and place, and could not answer questions in an intelligent manner. He showed also great fear when left alone. His blood Wassermann reaction was positive.



The spinal fluid cell count was 175 per c.mm., there was an excess of globulin and the Wassermann reaction was positive with 0.05 c.c.

He was given ten doses of mercurialized serum intraspinally and ten doses of salvarsan in addition to mercury inunctions and very large doses of potassium iodid by mouth. After five treatments a great change was noticeable in his condition. After his tenth treatment he was free from symptoms and seemed perfectly normal mentally.

There was no change in his blood Wassermann reaction. The Wassermann reaction in his spinal fluid changed from positive with 0.05 to positive with 0.2 c.c. The cell count fell from 175 per c.mm. to 9 per c.mm.

CASE 83.—A man, aged 38, was admitted to the hospital, Aug. 14, 1916. A diagnosis of tabes dorsalis was made. Eighteen years before he had had a chancre. For four years he had had infrequent attacks of nausea, with eructations of gas. For six weeks he had had constant pain in the epigastrium, which bore no relation to meals or time of day. He had become weak and tired and had lost 4 pounds in weight.

Physical examination showed unequal and irregular pupils, which did not react to light. The deep reflexes were unchanged. The Wassermann reaction in both blood and spinal fluid was positive. The spinal fluid gave a positive globulin test and showed a cell count of 52 per c.mm.

He was given three doses of mercurialized serum intraspinally and five intravenous doses of salvarsan. He has had no abdominal pain and no gastric symptoms since his second treatment.

The Wassermann reaction in the blood serum was not affected by treatment. The spinal fluid Wassermann reaction changed from positive with 0.2 c.c. to positive with 0.6 c.c. The spinal fluid cell count fell from 52 to 5 per c.mm.

CASE 84.—A woman, aged 47, entered the hospital Aug. 22, 1916. A diagnosis of general paralysis of the insane was made. She had had a syphilitic sore throat fifteen years before. For one year she had anorexia and vomiting after meals. For six months she had had headache, dizziness and increasing difficulty in walking. One week before entrance she had had a period of aphonia which lasted for nine hours. For several days she had had severe pains in the legs and a sense of constriction about her neck.

Physical examination showed tenderness to pressure over the whole skull. The pupils were unequal and did not react to light. The arm reflexes were very active. Knee and ankle jerks were diminished, but were present on both sides. There was marked swaying in the Romberg position. Gag reflex was absent.

The Wassermann reaction in both blood serum and spinal fluid was positive. The spinal fluid gave a positive globulin test and showed a cell count of 66 per c.mm.

Treatment consisted of five doses of mercurialized serum intraspinally, six doses of salvarsan intravenously, mercury and potassium iodid by mouth. After the second intraspinal treatment she claimed to be entirely free from symptoms.

The Wassermann reaction in the blood serum was unchanged. The spinal fluid Wassermann reaction changed from positive with 0.1 c.c. to positive with 0.5 c.c. The cell count fell from 66 to 20 per c.mm.

CASE 85.—A man, aged 48, entered the hospital Sept. 8, 1916. A diagnosis of general paralysis of the insane was made. He had had a chancre twenty years before. He had lost 30 pounds in the last five years. For one year he had had dizziness, nervousness and tremor of the muscles of his face.

Physical examination showed irregular pupils, which did not react to light. Knee jerks were present but unequal. He was dull mentally and had some memory defect.

Wassermann reaction in both blood serum and spinal fluid was positive. The spinal fluid gave positive globulin test and showed a cell count of 36 per c.mm.



He was given three doses of mercurialized serum intraspinally and four doses of salvarsan intravenously. There was no change in his condition from treatment. The Wassermann reaction in his spinal fluid changed from positive with 0.2 c.c. to positive with 0.3 c.c. The cell count fell from 36 to 5 per c.mm.

CASE 86.—A man, aged 35, entered the hospital Aug. 21, 1916. A diagnosis of *tabes dorsalis* was made. He had had gonorrhea twice, but denied having had syphilis. For nine years he had had paroxysmal attacks of pain in both shoulders, and for two years had had pain of the same kind in both legs, with parasthesias.

Physical examination showed irregular, unequal pupils, which did not react to light, and sluggish deep reflexes in both arms and legs.

The Wassermann reaction was positive in both blood and spinal fluid. The spinal fluid showed a cell count of 71 per c.mm. and gave a positive globulin test.

He was given four doses of mercurialized serum intraspinally and five doses of salvarsan intravenously. The Wassermann reaction was unchanged in his blood. The spinal fluid changed from positive with 0.2 c.c. to positive with 0.4 c.c. The cell count fell from 71 per c.mm. to 12 per c.mm. He was entirely relieved from pain and parasthesias after the third treatment.

CASE 87.—A man, aged 29, entered the hospital June 8, 1916. A diagnosis of rheumatic fever and *tabes dorsalis* was made. He had had rheumatic fever at the age of 18 and again at 26. He had had a chancre three years before entrance. He had had shooting pains in the back of his neck and head for six months.

Physical examination showed an acute arthritis of both knees and one shoulder. These manifestations promptly disappeared after a few days in the hospital. In addition he showed absent knee and ankle jerks, unequal and irregular pupils, which reacted poorly to light.

The Wassermann reaction in both blood and spinal fluid was positive. The spinal fluid showed a cell count of 11 per c.mm. and gave a positive globulin test.

He was given five doses of salvarsan intravenously without any change in symptoms or change in his spinal fluid cell count. After his first dose of mercurialized serum the cell count fell to normal. He had two additional doses of mercurialized serum intraspinally and six additional doses of salvarsan intravenously. His blood Wassermann reaction became negative and he became free from symptoms. The Wassermann reaction in his spinal fluid was unchanged.

CASE 88.—A woman, aged 28, entered the hospital Aug. 23, 1916. A diagnosis of cerebrospinal syphilis was made. She had had syphilis for four years. For two years she had had severe headaches and failing vision in her right eye, and for one year she had had nausea and vomiting whenever she had undertaken any physical exertion. For six months she had been very irritable, and for one month had been almost continuously in bed.

Physical examination showed unequal pupils, which did not react to light.

The Wassermann reaction was positive in both blood and spinal fluid. The spinal fluid cell count was 136 per c.mm.

She was given three doses of mercurialized serum intraspinally and six doses of salvarsan intravenously. This relieved her of all symptoms.

Her blood Wassermann reaction had not changed. The spinal fluid Wassermann reaction changed from positive with 0.3 c.c. to positive with 1.5 c.c. The spinal fluid cell count fell from 136 to 3 per c.mm.

CASE 89.—A woman, aged 48, entered the hospital June 27, 1916. A diagnosis of cerebrospinal syphilis was made. For two years she had had weakness of the legs and ataxia. For one year she had had headaches and for six weeks she had had such extreme headaches, dizziness and vomiting that she had been confined to bed.

Physical examination showed normal pupils, increased and unequal knee jerks, an ankle clonus on the right, and a positive Babinski's sign.

The Wassermann reaction in both blood and spinal fluid was positive. The spinal fluid cell count was 15. She was given three doses of salvarsan intravenously and two doses of mercurialized serum intraspinally. The blood Wassermann reaction was unchanged. The spinal fluid cell count fell from 15 to 6 per c.mm., and the Wassermann reaction changed from positive with 0.1 c.c. to positive with 0.4 c.c. This amount of treatment entirely relieved her of headaches and vomiting, the dizziness was greatly lessened and she was able to walk around without difficulty.

CASE 90.—A man, aged 42, entered the hospital March 4, 1916. A diagnosis of tabes dorsalis was made. For two years he had had numbness and weakness in his legs and for three months he had had unsteadiness in his gait.

Physical examination showed unequal pupils, which did not react to light, absent knee and ankle jerks, ataxia of the legs and a positive Romberg's test.

The Wassermann reaction in the blood serum was negative. The spinal fluid showed 107 cells per c.mm. and gave a positive Wassermann reaction.

He was given six doses of mercurialized serum intraspinally. Eight months after the treatment the numbness had disappeared from his legs and they became much stronger. Ataxia was lessened and he had gained 8 pounds weight.

CASE 91.—A man, aged 47, entered the hospital June 22, 1916. A diagnosis of general paralysis of the insane was made. He had had syphilis twenty-five years before. Five years before he had had a nervous breakdown and had become unsteady on his feet. For nine weeks he had had tenderness and pain in his left hand and for three weeks incontinence of urine.

Physical examination showed unequal pupils, which reacted poorly to light, exaggerated deep reflexes, a positive Romberg and a Babinski on each side. There was weakness and atrophy of the left hand, arm and leg, with disturbed sensation over the hand and arm.

The Wassermann reaction in the blood serum was negative. The spinal fluid showed a cell count of 34 per c.mm. and gave a positive Wassermann test with 0.5 c.c. He was given two intravenous doses of salvarsan and five intraspinal injections of mercurialized serum. The Wassermann reaction in his spinal fluid became more strongly positive under treatment, so that only 0.3 c.c. were required for complete fixation. The cell count fell to 4 per c.mm. There was little improvement in his condition clinically. He regained control of his urine and the pain in his hand became less severe.

CASE 92.—A woman, aged 48, entered the hospital March 20, 1916. A diagnosis of tabes dorsalis was made. She had had syphilis for eighteen years. For nine years she had had frequent pains in her legs. For six years she had had transient attacks of diplopia. She had had one dose of salvarsan and a great deal of mercury and potassium iodid without any relief.

Physical examination showed normal pupils and hyperactive deep reflexes in legs and arms.

The Wassermann reaction was negative in the blood. The spinal fluid showed 27 cells and gave a positive Wassermann reaction with 0.4 c.c.

She was given five doses of mercurialized serum intraspinally. The Wassermann reaction in the spinal fluid became negative, the cell count fell to 2 per c.mm. and she became entirely symptom-free.

CASE 93.—A man, aged 42, entered the hospital Aug. 31, 1916. A diagnosis of tabes dorsalis was made. For one year he had had difficulty in walking at night, urinary disturbance, sharp pains in his legs, occasional attacks of diplopia and rapidly increasing deafness. Eight months before he had fallen and broken his leg. He had lost 21 pounds weight in the last year.

Physical examination showed unequal, irregular pupils, which did not react to light, absent knee and ankle jerks, marked ataxia of hands and feet, double vision and almost total deafness.



The Wassermann reaction in the blood serum was negative. The spinal fluid showed 70 cells per c.mm. and gave a positive Wassermann test with 0.3 c.c.

He was given five doses of mercurialized serum intraspinally. The Wassermann reaction in the spinal fluid changed from positive with 0.3 c.c. to positive with 0.6 c.c. The cells fell from 70 to 7 per c.mm. There was no change in any of the patient's symptoms.

CASE 94.—A man, aged 48, entered the hospital May 15, 1916. A diagnosis of tabes dorsalis was made. He had had urinary disturbances for eighteen months and had lost 18 pounds weight during the same time. He had had pains in his legs for six months.

Physical examination showed unequal and irregular pupils, which did not react to light; unequal knee jerks; swaying in the Romberg position; tremor of the hands and some ataxia.

The Wassermann reaction in the blood serum was negative. The spinal fluid showed a cell count of 142 per c.mm. and gave a positive Wassermann reaction.

He was given seven doses of mercurialized serum intraspinally. The Wassermann reaction in his spinal fluid changed from positive with 0.2 c.c. to positive with 0.7 c.c. The spinal fluid cell count fell from 142 to 15 per c.mm. All his symptoms disappeared after the sixth treatment.

CASE 95.—A man, aged 42, entered the hospital May 6, 1916. A diagnosis of tabes dorsalis was made. For six years he had had lightning pains in his legs, which gradually became more severe and frequent. Mercury and potassium iodid by mouth had given no relief.

Physical examination showed irregular pinpoint pupils, which did not react to light; areas of hyperesthesia over the lower back; hyperactive reflexes in both arms and legs.

The Wassermann reaction in the blood was negative. The spinal fluid gave a positive Wassermann reaction with 0.5 c.c. and showed a cell count of 100 per c.mm.

He was given six doses of mercurialized serum intraspinally. The Wassermann reaction changed from positive with 0.5 c.c. to positive with 1.5 c.c. The cell count fell from 100 to 4 per c.mm. The pains were greatly lessened after only three treatments and after six treatments they almost entirely disappeared.

CASE 96.—A woman, aged 38, entered the hospital Aug. 4, 1916. A diagnosis of tabes dorsalis was made. One year before she had had both fallopian tubes removed because of gonococcus infection. For six months she had had attacks of sharp epigastric pain and vomiting coming on about once in three weeks. The attacks had been relieved only by morphin.

Physical examination showed irregular and unequal pupils, which reacted to light, a low, hypotonic stomach and a moderately dilated aorta. The Wassermann reaction in the blood was negative. The spinal fluid gave a cell count of 44 per c.mm.

She was given two doses of mercurialized serum intraspinally. The cell count in the spinal fluid was reduced from 44 to 0 per c.mm. by the first treatment. The Wassermann reaction was not changed. She had no pain or vomiting after treatment was begun.

CASE 97.—A man, aged 48, was admitted to the hospital Feb. 22, 1916. A diagnosis of tabes dorsalis was made. He had had a chancre twenty-six years before. He had been treated for five years for chronic nephritis. For two years before admission he had had difficulty in walking, especially at night, and dizziness. These symptoms had grown worse rapidly, so that for three months he had been almost continually in bed.

Physical examination showed a moderately advanced chronic nephritis with a systolic blood pressure of 210 mm. of mercury. The pupils were irregular and reacted poorly to light. The knee jerks were hyperactive.



The Wassermann reaction in his blood serum was negative. The spinal fluid showed a cell count of 6 per c.mm. and gave a positive Wassermann reaction with 0.3 c.c.

He was given four doses of mercurialized serum intraspinally. The Wassermann reaction in the spinal fluid became negative with 1 c.c. and the cell count fell to 2 per c.mm. After three treatments the dizziness entirely disappeared and he became able to resume his work, which he had not touched for more than two years.

CASE 98.—A man, aged 61, entered the hospital May 24, 1916. A diagnosis of tabes dorsalis was made. For ten years he had had paresthesias of his feet and for two years difficulty in walking, with pains in his legs and thighs.

Physical examination showed Argyll Robertson pupils and absent knee and ankle jerks.

The Wassermann reaction in the blood was negative. The spinal fluid gave a positive reaction with 0.4 c.c., and showed a cell count of 200 per c.mm.

He was given two doses of mercurialized serum intraspinally. The pains in his legs were lessened. The cell count in the spinal fluid fell from 200 to 10 per c.mm. The Wassermann reaction was unchanged.

CASE 99.—A woman, aged 41, entered the hospital Jan. 15, 1916. A diagnosis of general paralysis of the insane was made. She had had syphilis for fourteen years. For three years she had had sharp pains in her extremities, and had lost 18 pounds weight during that time. She had had mercury continuously for six months without any benefit.

Physical examination showed irregular, fixed pupils and unequal knee jerks. She was very talkative and had some memory defect.

The Wassermann reaction in the blood serum was negative. The spinal fluid gave a positive reaction with 0.1 c.c. and showed a cell count of 20 per c.mm. She was given two doses of mercurialized serum intraspinally. The cell count fell to 6 per c.mm. and the Wassermann reaction changed from positive with 0.1 c.c. to positive with 0.5 c.c. All the pains disappeared after the first treatment and did not recur. There was no change in her mental condition.

CASE 100.—A man, aged 50, entered the hospital Sept. 12, 1916. A diagnosis of tabes dorsalis was made. For eight years he had had occasional attacks of epigastric pain, nausea and vomiting. He had had difficulty in walking in the dark for seven years. For six months he had had girdle sensation and pains in his legs.

Physical examination showed unequal irregular fixed pupils; absent knee and ankle jerks; positive Romberg's test and ataxia of both hands and feet.

The Wassermann reaction was negative in the blood and spinal fluid. The spinal fluid also gave a negative globulin test and showed only 8 cells per c.mm. He was given two doses of mercurialized serum intraspinally which caused no change in symptoms, but a rise in the spinal fluid cell count to 74 per c.mm. Two months later the cell count had fallen to 12 per c.mm. and he stated that his pains and girdle sensation had disappeared.

CASE 101.—A man, aged 53, entered the hospital Sept. 7, 1916. A diagnosis of tabes dorsalis was made. He had had attacks of severe abdominal pain with vomiting almost every week for three months.

Physical examination showed Argyll Robertson pupils.

The Wassermann reaction in the blood was positive. The spinal fluid showed a cell count of 56 per c.mm. and gave a positive Wassermann reaction with 0.2 c.c. He was given three doses of mercurialized serum intraspinally and four doses of salvarsan intravenously. The Wassermann reaction in the blood serum was unchanged. The spinal fluid Wassermann reaction was changed from positive with 0.2 c.c. to positive with 0.6 c.c. The cell count fell from 56 to 5 per c.mm. The attacks of vomiting still continue but are not of such duration or frequency as before treatment.

Eleven patients previously treated with salvarsanized serum were each given three or more intraspinal treatments with mercurialized serum, a total of thirty-eight doses. The effect on the Wassermann reaction, cell count and clinical symptoms was in every case equally as good as, if not better than, from the same amount of salvarsanized serum. The reactions in every case were more severe than with the salvarsanized serum.

Peter Bent Brigham Hospital.

## THE SALICYLATES

### VI. RENAL, FUNCTIONAL AND MORPHOLOGIC CHANGES IN ANIMALS FOLLOWING THE ADMINISTRATION OF SALICYLATE \*

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It was previously shown<sup>1</sup> that individuals receiving full therapeutic doses of salicylate invariably have albumin, leukocytes and casts or cast-like bodies in the urine. This albuminuria is produced in both febrile and afebrile individuals, and is of renal origin. The functional tests (phenolsulphonephthalein excretion in urine and nonprotein nitrogen of the blood) which were applied at the time, gave inconstant results, and the changes in some cases were so small that it was impossible to conclude definitely whether any serious impairment in the functional efficiency of the kidney existed. It is possible that repeated administration of salicylate might cause more definite and marked changes in the kidney, both functionally and morphologically. Moreover, in certain individuals, the albuminuria and other changes are so severe that the presence of a definite nephritis is suggestive.

Von Ackeren<sup>2</sup> gave sodium salicylate and salicylic acid per os to five rabbits, and two of these animals showed "acute nephritis," with albuminuria and hematuria. He cites fourteen cases in the literature up to 1890, in which there was albuminuria, sometimes accompanied by hematuria, following administration of salicylate. Vinci<sup>3</sup> observed a toxic parenchymatous nephritis at the necropsy of a man 45 years of age, who died thirty-one hours after the ingestion of 35 gm. of sodium salicylate. There was marked congestion of all the renal vessels, particularly in the glomerular tuft, albuminous precipitate in the subscaplar space, interstitial hemorrhage, cloudy swelling and extensive necrosis of the tubular epithelium, most marked in the cortex;

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1. Scott, R. W., and Hanzlik, P. J.: The Salicylates: III. Salicylate Albuminuria, *Jour. Am. Med. Assn.*, 1916, **67**, 1838.

2. Von Ackeren, A.: Ueber Nierenreizung nach Salicylsäure Gebrauch, *Charité Ann.*, 1890, **15**, 252.

3. Vinci, G.: Sulle lesioni istologiche sperimentali del rene determinate dall'acido salicilico con un caso raro di avvelenamento nell'uomo salicilato di soda, *Arch. di farmacol. sper.*, 1905, **4**, 59.



but as the necropsy was performed two days after death, June 25, and as the kidneys sent to Vinci were imperfectly preserved in alcohol, little importance can be attached to the histologic findings. Experimenting with dogs, guinea-pigs and rabbits, he found that massive single doses, and frequently repeated large doses, led essentially to the same change as he described in the human kidney. For the most part the drug was given by mouth, but in a few instances was given intravenously or subcutaneously. He states that the lesions were most marked in dogs, less in guinea-pigs and least in rabbits. Lüthje,<sup>4</sup> in addition to a large amount of clinical work, gave repeated doses of sodium salicylate to two dogs, and both showed cylindruria, one showing albuminuria. Busse examined the kidneys and found general congestion, interstitial hemorrhage, cloudy swelling, fatty degeneration, cast formation, and in the subscapular space of the glomerulus a homogeneous coagulum. With the exception of the reports of von Ackeren, Lüthje and Vinci, the literature seems to be devoid of carefully controlled experimental work on the effect of salicylate on the kidneys of lower animals.

The object of this study is to ascertain what effect the administration of salicylate has on the morphology of the kidney and its functional efficiency as judged by certain nitrogenous constituents of the blood in animals subjected to varying conditions of dosage, mode and duration of administration of the drug. Our own work was carried out on dogs, cats and one rabbit. The dosage of salicylate used corresponded to the so-called "toxic" or full therapeutic dose used with human individuals suffering with rheumatic fever, that is, about 0.23 gm. of sodium salicylate (0.2 gm. salicylic acid) per kilo. In some cases only one such dose was administered. In other cases, repeated doses were used with the object of producing a nephritis if possible. Usually, the salicylate (10 per cent. solution in water) was administered hypodermically. In a few instances the drug was also administered by mouth, but oral administration was not generally practiced because of loss of the drug by emesis.

As a rule, the animals were observed for two to three days before the experiment was begun, since they almost invariably showed some evidences of albuminuria. The drug was then injected and careful notes of symptoms, urinary and blood changes were made. Albumin was tested for qualitatively by the heat and acetic acid and ferrocyanid tests. The blood for nitrogen estimations was obtained directly from the heart. Nonprotein nitrogen was estimated according to the method

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4. Lüthje, H.: Ueber die Wirkung von Salicylpräparaten auf die Harnwege nebst einigen Bemerkungen über die Genese der Cylinder und Cylindroide, *Deutsch. Arch. f. klin. Med.*, 1902, **74**, 163.

of Folin and Denis,<sup>5</sup> and urea nitrogen by the urease method, using an aqueous extract of jack bean preserved with glycerin,<sup>6</sup> aeration and nesslerization. A microscopic examination of the urine was made regularly for the presence of leukocytes, erythrocytes and casts. In a number of animals the reaction of the blood and the reserve alkalinity before and after the administration of salicylate were estimated according to the methods of Levy, Rowntree and Marriott<sup>7</sup> (reaction of blood) and Marriott<sup>8</sup> (alkali reserve).

The animals were usually killed by a few whiffs of chloroform on the expiration of a given time; necropsy was performed and the kidneys were excised. Small blocks from both organs (about 5 mm. thick) were fixed immediately in Zenker's fluid and in formaldehyd, embedded in celloidin and stained with hemalum and eosin.

The various pertinent data have been summarized in the accompanying table. For further details, the appended brief protocols of all the experiments may be consulted.

#### DISCUSSION

The data in the table and protocols indicate that the administration of salicylate causes the appearance of albumin, leukocytes, granular casts or cast-like bodies, and sometimes red blood corpuscles in the urine of cats, dogs and one rabbit. When these elements were present before the experiment they were increased by the administration of the drug. This is confirmatory (except appearance of erythrocytes in urine) of what was observed in human beings.

Practically all (ten) of the eleven animals, whose blood was examined, showed a variable though definite accumulation of nonprotein and urea nitrogen, an indication, therefore, of diminished renal functional efficiency so far as these tests are concerned. In Dog 17 certain of the results for urea nitrogen appear rather low. However, repeated determinations confirmed the results. There seems to be, at least in certain individuals, an increase in renal permeability for urea at an early stage in the action of salicylate as indicated by these figures. This phenomenon was previously observed, and apparently is more commonly encountered in human beings. It was partly because of

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5. Folin, O., and Denis, W.: New Methods for the Determination of Total Nonprotein Nitrogen, Urea and Ammonia in Blood, *Jour. Biol. Chem.*, 1912, **11**, 527.

6. For this method of preparation of urease we are indebted to Dr. Cyrus H. Fiske of the Biochemical Laboratory.

7. Levy, R. L., Rowntree, L. G., and Marriott, W. McK.: A Simple Method for Determining Variations in the Hydrogen-ion Concentration of the Blood, *THE ARCHIVES INT. MED.*, 1915, **16**, 389.

8. Marriott, W. McK.: A Method for the Determination of the Alkali Reserve of the Blood Plasma, *THE ARCHIVES INT. MED.*, 1916, **17**, 840.



this variability of the effect, that is, function seemed to be favored sometimes, other times diminished, and partly because of the small differences in the results obtained, that it was impossible to decide as to the extent of impairment in kidney function. The study of additional human cases seems to confirm what certainly appears to be the case in animals, namely, a diminution in renal functional efficiency. Just how permanent this change is cannot be definitely stated. In two of the animals there was a tendency for the urea nitrogen of the blood to return to the previous level, and in the majority of those observed over long periods of time the albuminuria gradually diminished.

So far as the reaction and the reserve alkalinity of the blood are concerned, no important changes were observed in the few animals studied.

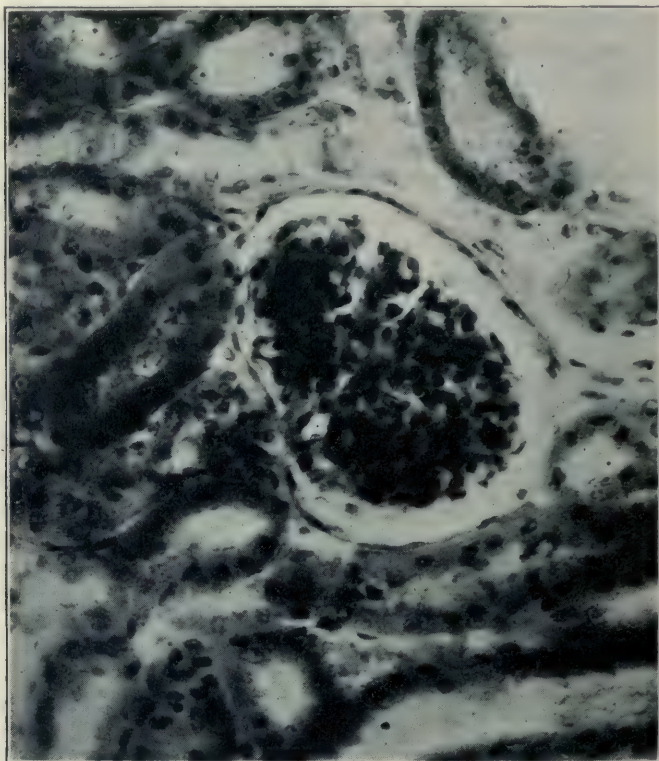
As to morphologic changes, the data indicate that full therapeutic doses of sodium salicylate are capable of producing in animals definite lesions of the kidney, easily demonstrable by microscopic examination. In the case of Animal 17, oral administration of the drug produced a renal lesion quite as severe, both functionally and morphologically, as followed subcutaneous administration.

Considering the series as a whole, the administration of the drug was followed by histologic lesions varying from slight cloudy swelling of the tubular epithelium to severe cloudy swelling associated in a few cases with necrosis and desquamation, and in nine instances with distinct glomerular lesions. The glomerular lesions were never very severe and consisted principally of swelling and multiplication of capillary endothelium. In these cases the severe involvement of the tubules seemed to justify emphasizing that phase in the diagnosis rather than making a diagnosis of acute glomerular nephritis. The finding of albuminous granular precipitate in the subcapsular space, in the opinion of the writers, does not justify a definite diagnosis of glomerular lesion; accordingly several cases diagnosed as cloudy swelling show in addition this minor change.

The proximal convoluted tubule was apparently the first part attacked followed very rapidly by lesion of the distal convoluted tubule, then of the ascending limb of the loop of Henle. As a rule, it appeared that these divisions were involved before lesion of the glomerulus occurred. In cases in which all these features appeared, the lesion of the proximal convoluted tubule was the most severe change. No stress is laid on fatty changes, because, independently of experimental procedures, such changes are practically constant in the cat and frequent in both the dog and rabbit. The diagnosis of necrosis of tubular epithelium in these kidneys rests in part on finding distinct nuclear pyknosis, and in part on finding desquamation of entire cells and groups of cells. The accompanying figure illustrates a renal lesion.



Although Vinci found differences in the susceptibility of dogs, guinea-pigs and rabbits, the experiments reported in this paper show no material differences in susceptibility of dogs and cats. He found essentially the same type and distribution of tubular lesion as we have described, but in contrast to the definite intracapillary glomerulitis seen in some of our animals, he describes only marked congestion of the tuft. In his series and ours there was found cloudy swelling of glomerular epithelium and albuminous material in the subcapsular



Kidney of Dog 12 showing slight involvement of the glomerular tuft, which, although not enlarged, is richly cellular and devoid of blood. The tubular epithelium shows cloudy swelling and the lumina contain granular albuminous precipitate.

space. Vinci's failure to find the more marked glomerular lesion is probably due to the fact that most of his animals died or were killed after only seven or eight hours.

It is not possible in this series definitely to correlate the morphologic and functional changes. It will be noted as an indication of this difficulty that the most marked accumulation of urea nitrogen occurred in connection with a kidney that histologically showed no change in the

glomeruli except cloudy swelling of the lining epithelium and albuminous precipitate in the subcapsular space (Experiment 4).

An interesting fact, which may or may not have a bearing on the problem of albuminuria and nephritis, is a diminution in the total excretion of salicylate in the urine of Dog 18 as compared with the urinary excretion of Dog 19. The kidney of Dog 18 shows a definite, acute tubular nephritis which, in part, at least, may have been present before the animal received salicylate. Dog 19 was in good condition and the kidneys show cloudy swelling without glomerular change. Relatively, the accumulation of urea nitrogen in the blood of Dog 18 is also greater than that of Dog 19. Whatever the explanation may be, it is nevertheless interesting to note that the animal showing the more profound renal functional and morphologic changes excreted less salicylate than the animal with the less pronounced changes. This coincides with certain observations reported by Hanzlik, Scott and Thoburn<sup>9</sup> on the excretion of salicylate in human beings with different clinical conditions as compared with normal individuals.

#### CONCLUSIONS

1. The administration of salicylate in doses corresponding to full therapeutic doses for human beings per kilo of body weight, causes the appearance of albumin, leukocytes, casts or cast-like bodies, and sometimes red blood corpuscles, in the urine of animals (cats, dogs and one rabbit).

2. A pre-existing albuminuria is aggravated by the administration of salicylate.

3. The albuminuria is of direct renal origin.

4. So far as the nonprotein and urea nitrogen of the blood are concerned, there is a diminution in renal functional efficiency.

5. Morphologically, a lesion of the kidney appears, varying in severity from simple cloudy swelling of the epithelium of the proximal convoluted tubule to extensive cloudy swelling of all the cortical parts of the tubules, associated with an acute intracapillary glomerulitis, the latter process being denominated as an acute tubular nephritis.

#### PROTOCOLS

EXPERIMENT 1.—Dog; 3.1 kg. Urine before salicylate was albumin-free; received 0.3 gm. sodium salicylate per kilo by mouth; 1 hour later there was marked albuminuria. On the following day there was still marked albuminuria; received two doses of 0.3 gm. each per kilo, but vomited almost immediately after each dose; 4 gm. of salicylate were then injected in three divided doses

9. Hanzlik, P. J., Scott, R. W., and Thoburn, T. W.: The Salicylates. V. Secretion of Salicyl in the Urines of Rheumatic and Non-Rheumatic Individuals, *Jour. Pharm. and Exper. Therap.*, 1917, **9**, 247.



hypodermically within thirty minutes; animal was killed 1½ hours after last dose. Albuminuria was severe; red and white cells were present in the urine.

Histologically the glomeruli show slight congestion, but otherwise normal tufts; occasional glomeruli show albuminous precipitate in the subcapsular space. Epithelium of the proximal convoluted tubules shows moderate cloudy swelling; the lumina of these tubules are normal. There are no casts.

Diagnosis: Cloudy swelling of kidney.

EXPERIMENT 2.—Dog; 5.4 kg. Urine before salicylate contained traces of albumin, which was markedly increased, together with the appearance of white and red cells after the subcutaneous injection of 0.9 gm. sodium salicylate. Two days later the animal received 3.3 gm. salicylate in two divided doses. The animal died during the night; urine from bladder gave strong albumin tests and showed the presence of white and red corpuscles.

Histologic picture somewhat obscured by postmortem degeneration. Glomerular tufts normal save for slight congestion; the subcapsular spaces contain much granular precipitate and also a moderate number of desquamated capsular epithelial cells; this latter condition, however, is probably the result of postmortem changes. The epithelium of the convoluted tubules shows marked granularity and the lumina contain many albuminous granules. There are no casts. Other tubules show less marked granular change. Around some of the large blood vessels there is an old connective tissue growth.

Diagnosis: Postmortem degeneration and probably cloudy swelling of kidney.

EXPERIMENT 3.—Dog; 5.6 kg. For four days previous to injection of salicylate, the urine showed a trace of albumin. On day of experiment the urine was albumin-free. Received single injection of 0.05 gm. salicylate per kilo hypodermically. Seven hours later the urine gave strong albumin tests and showed the presence of white and red corpuscles; dog found dead in cage next morning; 5 c.c. of urine obtained from the bladder; this gave strong albumin tests, and showed numerous white corpuscles and granular cast-like bodies.

Postmortem degeneration has advanced to such a degree as to render histologic description useless.

EXPERIMENT 4.—Cat; 3.2 kg. Urine before salicylate showed questionable trace of albumin for two days; 1.05 gm. sodium salicylate was injected in three divided doses in the course of forty-five hours; 700 c.c. water were administered at different times and about 300 c.c. urine were voided during this interval. The animal was killed. The urine at all times gave marked albumin tests and showed many white, and a few red corpuscles.

Histologically the glomeruli show practically normal vascular tufts; the subcapsular space in many instances shows a moderate amount of albuminous precipitate; a small number of glomeruli show swelling of the capsular epithelium. The epithelium of the proximal convoluted tubules show well marked granularity of the protoplasm, in some instances hyaline droplet formation, and infrequently many desquamated epithelial cells. The tubules otherwise are normal; there are no casts.

Diagnosis: Cloudy swelling of kidney. The change might be called by some authors an acute tubular nephritis, but without more marked tubular change, and especially in the absence of notable change in the glomerular tuft, such a diagnosis seems hardly justifiable.

EXPERIMENT 5.—Cat; 3 kg. Trace of albumin present in urine for a week before salicylate; 0.9 gm. sodium salicylate injected in two successive days; total injected 1.8 gm. Following second injection, marked albuminuria, few granular casts; many leukocytes, few erythrocytes; animal was killed in a dying condition on the second day.

Histologically the interstitial tissue is normal. The glomeruli show normal capsules and no exudate in the subcapsular space, but the vascular tufts are large, fill the capsular spaces and are almost devoid of blood. The epithelium



of the cortical tubules contains much fat, but marked granularity of the protoplasm can easily be made out, and the lumina of many of the tubules contain fine and coarsely granular albuminous precipitate.

Diagnosis: Acute tubular nephritis.

EXPERIMENT 6.—Cat; 2.1 kg. Trace of albumin in urine before salicylate; 0.6 gm. sodium salicylate injected in two doses within twenty-six hours; total injected 1.2 gm. Marked albuminuria together with granular casts and leukocytes. Animal markedly depressed; killed.

Histologically, the interstitial tissue and the glomeruli are in all respects normal. The epithelium of proximal and distal convoluted tubules, as well as of the ascending loop of Henle, show various stages of cloudy swelling, from swelling of the epithelium with occlusion of the lumen to narrowing of the epithelial lining, with the presence of a considerable amount of granular precipitate in the lumina. There are a few hyaline casts and a few minute interstitial hemorrhages.

Diagnosis: Slight cloudy swelling; almost a normal kidney.

EXPERIMENT 7.—Rabbit; 1.2 kg. Urine free from albumin before salicylate; 2.2 gm. sodium salicylate injected in four doses on three successive days. Following each injection, mild albuminuria, together with occasional granular and hyaline casts. Throughout the experiment the animal appeared normal; killed.

Histologically, the interstitial tissue shows numerous small areas of overgrowth with associated atrophy of tubules and glomeruli. In the large areas between these foci the interstitial tissue is apparently normal. The glomerular capsules and subcapsular spaces show nothing pathologic. The vascular tufts are large, bloodless and apparently the seat of an acute glomerulitis. In somewhat irregularly distributed areas the convoluted tubules show marked granularity, vagueness of outline, fragmentation and desquamation, with slight pyknosis of a few of the nuclei. The tubular lumina contain granular albuminous precipitate and a few hyaline casts.

Diagnosis: Acute tubular nephritis superimposed on chronic interstitial nephritis.

EXPERIMENT 8.—Cat; 1.9 kg. Slight albuminuria present before salicylate. A single injection of 0.6 gm. sodium salicylate was given. Animal found dead in cage next morning; bladder urine contained large quantity of albumin and casts.

Histologically, the interstitial tissue and the glomeruli are normal, except that a very few of the latter show large, bloodless, richly cellular, vascular tufts. The epithelium of the cortical tubules is more granular than normal and occupies a decreased space in the tubule. The correspondingly enlarged lumina are filled with finely and coarsely granular albuminous precipitate. Several arched collecting tubules contain hyaline casts.

Diagnosis: Acute tubular nephritis.

EXPERIMENT 9.—Cat; 1.3 kg. Trace of albumin in urine before salicylate; 0.85 gm. sodium salicylate injected in two doses on two successive days. Animal died in 1½ hours after second injection; kidneys excised immediately after death; bladder urine gave strong tests for albumin; granular casts present.

Histologically, the interstitial tissue is normal, as are also the glomerular capsules and subcapsular spaces. The glomerular tufts are large, devoid of blood and richly cellular. The epithelium throughout the cortex shows cloudy swelling, but in the proximal and distal convoluted tubules the granulation is very marked; there is fragmentation and desquamation of cells, the tubular lumina are filled with albuminous precipitate and an occasional narrow hyaline cast can be seen.

Diagnosis: Acute tubular nephritis.

EXPERIMENT 10.—Dog; 6.5 kg. Urine free from albumin and casts before salicylate; 6.05 gm. sodium salicylate injected in four divided doses on four

TABLE SHOWING EFFECT OF SALICYLATE ON KIDNEY<sup>1</sup>

Number and Animal	Body Weight, Kg.	Total Quantity of Sod. Salicylate <sup>2</sup> Administered, Gm.	No. of Toxic Doses	Mode of Administration	Albuminuria		N.P.N. and Urea-N in Blood (Mg. in 100 C.c.)		Reaction of Blood (pH) and Alkali Reserve (RPH)		Pathologic Diagnosis	Experiment Terminated End of
					B. S.	A. T.	B. S.	A. T.	B. S.	A. T.		
Dog 1	3.1	4.6	6	Hypo + per os	+st	+st	.....	.....	.....	.....	Cloudy swelling	2 days
Dog 2	5.4	4.2	3	Hypo.	+tr	+st	.....	.....	.....	.....	Cloudy swelling (?)	3 days
Dog 3	5.6	0.05	1	Hypo.	—	+	N.P.N. 52.0	62.0	.....	.....	.....	2 days
Cat 4	3.2	1.05	3	Hypo.	+?	+	N.P.N. 22.4	130.4	.....	.....	Cloudy swelling	45 hours
Cat 5	3.0	1.8	2	Hypo.	+tr	+	.....	.....	.....	.....	Acute tubular nephritis	3 days
Cat 6	2.1	1.2	2	Hypo.	+sl	+st	.....	.....	.....	.....	Very slight cloudy swelling	26 hours
Rabbit 7	1.2	2.2	4	Hypo.	+sl	+st casts	.....	.....	.....	.....	Acute tubular nephritis superimposed on chronic interstitial nephritis	3 days
Cat 8	1.9	0.6	1	Hypo.	+sl	+st	.....	.....	.....	.....	Acute tubular nephritis	20 hours (?) (Died during night)
Cat 9	1.3	0.85	2	Hypo.	+sl	+st	.....	.....	pH 7.4	pH 5.4	Acute tubular nephritis	20 hours
Dog 10	6.5	6.05	4	Hypo.	—	+	N.P.N. 20.0	(1) 40.2 (2) 49.0 (3) 66.7 (4) 80.0	.....	.....	(No sections)	4 days

Dog 11	6.3	2.19	3	Hypo.	+sl	+	Urea N 24.5	(1) 24.2 (2) 18.0 (3) 40.3	pH 7.6	pH 7.7 7.6 7.6	Cloudy swelling	3 days
Dog 12	5.5	3.5	2	Hypo.	+vsl	+	Urea N 6.03	(1) 6.2 (2) 8.73 (3) 18.2	pH 7.7	pH 8.0	Cloudy swelling	3 days
Cat 13	2.1	0.94	1	Hypo.	+	+	Urea N 20.4	20.3	pH 7.6 RpH 8.2	pH 7.6 RpH 8.2	Acute tubular nephritis	26 hours
Cat 14	0.5	0.15	1	Hypo.	Nourine	Nourine	Urea N 12.8	13.7	.....	.....	Acute tubular nephritis	6½ hours
Cat 15	0.5	0.15	1	Hypo.	Nourine	Nourine	Urea N 16.1	.....	.....	.....	Slight cloudy swelling	6½ hours
Cat 16	2.0	0.6	1	Hypo.	+sl	+ casts	Urea N 20.1	25.8	.....	.....	Acute tubular nephritis	9 hours
Dog 17	7.5	3.0	2	Per os	+	+	Urea N 8.56	(1) 4.1 (2) 3.3 (3) 6.2 (4) 12.3 (5) 12.8 (6) 18.0 (7) 18.2 (8) 19.3 (9) 14.0	pH 7.8 RpH 8.4	pH 7.7 RpH 8.2 7.8 8.2 7.9 8.5 7.7 8.4 7.6 8.4 7.6 8.6 7.6 8.4	Acute tubular nephritis	9 days
Dog 18 <sup>a</sup>	4.0	1.2	1	Hypo.	+	+	Urea N 7.1	(1) 12.8 (2) 12.8 (3) 16.0 (4) 18.6	.....	.....	Acute tubular nephritis	4 days
Dog 19 <sup>a</sup>	5.8	1.3	1	Hxpo.	-	+	Urea N 11.0	(1) 14.0 (2) 14.1 (3) 9.5 (4) 10.0	.....	.....	Marked cloudy swelling	4 days

1. The abbreviations in the table have meanings as follows: B. S. = before salicylate; A. T. = at "toxicity"; or when the effects of the salicylate were evident as indicated by symptoms of nausea, vomiting, loss of equilibrium, etc.; N.P.N. = nonprotein nitrogen; pH = hydrogen-ion concentration; RpH = reserve alkalinity; st = strong; sl = slight; + = present; - = absent; figures in parentheses refer to days.
2. Sodium salicylate contains 86 per cent. salicylic acid.
3. Total quantity of salicyl recovered in urine = 28.6 per cent. The distillation-colorimetric method was used as described by Thoburn and Hanzlik: Jour. Biol. Chem., 1915, 23, 163.
4. Total quantity of salicyl recovered in urine = 55.4 per cent.



successive days. Within one to two hours after each of the first two injections the dog was depressed, with increased respiration. On the day of the third injection the animal appeared normal and in good condition, but following the third and fourth injections of the drug the animal became progressively more depressed and weak and died sometime during the following night and day (over Sunday). Albuminuria, together with leukocytes and considerable epithelium, appeared following the first injection and progressively increased until death (at times red corpuscles were present). Kidneys not sectioned because of postmortem changes.

EXPERIMENT 11.—Dog; 6.3 kg. Mild albuminuria together with leukocytes present before salicylate. Sodium salicylate was injected in three divided doses on three successive days as follows; 0.3 gm. daily for the first two days, and 1.89 gm. on the third day; total injected, 2.19 gm. Dog vomited and showed considerable depression after each injection. Albuminuria, together with casts, leukocytes and epithelium, progressively increased until the animal was killed on the third day. Blood was obtained from the heart daily for urea nitrogen determination; pericardium and myocardium appeared normal.

Histologically, the interstitial tissue and glomeruli are normal. The cortical tubules show a narrowed epithelial lining, the cells of which show increased granularity and fragmented, ragged edges; the tubular lumina are well filled with granular albuminous precipitate.

Diagnosis: Cloudy swelling.

EXPERIMENT 12.—Dog; 5.5 kg. Faint trace of albumin in urine before salicylate; 3.5 gm. sodium salicylate injected in two doses on successive days. Considerable depression and vomiting followed second injection. Marked albuminuria, together with granular casts, leukocytes, some erythrocytes and clumps of epithelium present in urine. Animal killed.

Histologically, the interstitial tissue is normal, as also are the glomeruli, except for a slight deposit of granular albuminous material in the subcapsular space. The epithelium of the cortical tubules is more granular than normal and the tubular lumina, increased in caliber, contain much albuminous granular precipitate.

Diagnosis: Cloudy swelling.

EXPERIMENT 13.—Cat; 2.1 kg. Albuminuria present before salicylate; 0.94 gm. sodium salicylate injected as follows: 0.62 gm. on the first day, 0.32 gm. on the second day. Convulsions of medullary type occurred about eight hours after first injection, and cat died in about two hours after the second injection. Albumin in urine present after each injection, though not marked.

Histologically, the interstitial tissue is normal. The glomerular tufts are large, richly cellular and bloodless. The convoluted tubules show a moderate amount of fat. The cortical tubules as a whole show marked granularity of cell protoplasm, in several places, desquamation. The tubular lumina contain albuminous granular material and several of the distal convoluted tubules show an accumulation of hyaline material in the lumina, not as yet fused and separated to form casts.

Diagnosis: Acute tubular nephritis.

EXPERIMENT 14.—Kitten; 0.5 kg. No urine obtainable at any time. Single dose of 0.15 gm. sodium salicylate injected. Animal remained in good condition; killed at the end of 6½ hours after injection.

The histologic changes in this kidney are practically the same as those seen in Animal 13.

Diagnosis: Acute tubular nephritis.

EXPERIMENT 15.—Kitten; 0.5 kg. No urine obtainable at any time. Single dose of 0.15 gm. sodium salicylate injected. Considerable depression at the end of six hours after injection; died while attempting to secure blood from heart.

Histologically, the interstitial tissue is normal. The glomeruli are large, contain a moderate amount of blood and there is a small amount of granular albuminous precipitate in the subcapsular spaces.

The cortical tubular epithelium shows slightly increased granularity and the tubular lumina contain a small amount of granular albuminous material.

Diagnosis: Slight cloudy swelling.

EXPERIMENT 16.—Cat; 2 kg. Slight albuminuria, together with leukocytes before salicylate. Single dose of 0.6 gm. sodium salicylate injected. Albuminuria considerably increased; leukocytes numerous; some erythrocytes and cast-like bodies. Animal killed at end of nine hours after injection.

Histologically, the interstitial tissue is normal. The vascular tufts of the glomeruli are large, contain an occasional erythrocyte, but are more richly cellular than normal and frequently show filling of capillary loops by endothelium. The subcapsular spaces contain albuminous granular precipitate. The cortical tubules show well marked cloudy swelling, most marked in the proximal convoluted tubules, where the cells are large and extremely granular, and the tubular lumina are filled with much albuminous granular precipitate.

Diagnosis: Acute tubular nephritis.

EXPERIMENT 17.—Dog; 7.5 kg. Albuminuria present, but no casts before salicylate. On the first day 0.25 gm. sodium salicylate was administered into the stomach every hour until six doses were given; total salicylate administered, 1.5 gm. Urine was expressed from the bladder at intervals, but contained no more albumin than before salicylate; no casts; occasional leukocytes and erythrocytes present; no vomiting; no depression. On the second day 1.5 gm. salicylate were administered in the same way as on the first day; total salicylate administered in experiment, 3 gm. Vomiting occurred after the last dose was administered; the animal showed some depression and incoordination; more vomiting occurred during the following night. The animal was observed continuously for seven days after the last injection; total observation period, nine days. Albuminuria increased gradually up to the fourth day after injection of salicylate, then gradually diminished until the last day, when only a fairly positive trace of albumin was present; no casts at any time. The animal gained weight slightly (160 gm.) and remained in good condition throughout the experiment. Blood from the heart was obtained daily for urea nitrogen and reaction estimations; killed on the ninth day.

Histologically, the interstitial tissue is normal. The vascular tufts of the glomeruli are large, bloodless and richly cellular. The subcapsular spaces contain granular albuminous precipitate. The cortical tubules show cloudy swelling, most markedly in the proximal convoluted tubules, where there is marked granularity of the epithelium as well as fragmentation and desquamation. The tubular lumina show much granular albuminous material and a few epithelial and coarsely granular casts.

Diagnosis: Acute tubular nephritis.

EXPERIMENT 18.—Dog; 4 kg. Albumin present (more than a trace) and some leukocytes before salicylate. A single dose of 1.2 gm. sodium salicylate (actually 1.32 gm. salicylic acid) was injected hypodermically. Animal observed for next four days. Dog originally was not very vigorous; no depression from salicylate, but progressively became morose, emaciated and emitted a foul odor from the oral cavity, which showed signs of infection; site of salicylate injection was edematous and necrotic. Albuminuria, together with leukocytes, present throughout experiment, and remained about the same after the salicylate. Blood was secured from the heart daily for urea nitrogen estimation; killed on fourth day. Pericardium and myocardium appeared normal. Left kidney showed about ten large, irregular white patches diffusely scattered on the surface; in some places these appeared to be raised, resembling miliary tubercles. The right kidney showed three such patches. Other viscera appeared normal. The

total quantity of salicylate recovered from the cage contents (urine, etc.) was 26.6 per cent., expressed as salicylic acid.

Histologically, the interstitial tissue is normal. The glomeruli show large, bloodless, richly cellular vascular tufts and a small amount of finely granular albuminous precipitate in the subcapsular space. The cortical tubular epithelium shows cloudy swelling, most markedly in the proximal convoluted tubules, where there is marked granularity, fragmentation and desquamation, the tubular lumina containing granular albuminous material but no casts.

Diagnosis: Acute tubular nephritis.

EXPERIMENT 19.—Dog; 5.8 kg. Urine practically free from albumin before salicylate. Single dose of 1.3 gm. sodium salicylate (actually 1.235 gm. salicylic acid). Animal became nauseated, but no vomiting occurred; for two days following the injection, the albuminuria increased, then gradually disappeared, only a trace being present on the last day of the experiment. Blood was secured daily from the heart for urea nitrogen estimation; the animal remained in good condition; killed on the fourth day; all viscera appeared normal. The total quantity of salicylate recovered from the cage contents (urine, etc.) was 55.4 per cent., expressed as salicylic acid.

Histologically, the interstitial tissue is normal. The glomeruli are normal, except that the subcapsular spaces frequently contain granular albuminous precipitate. The cortical tubular epithelium, as in Dog 18, shows cloudy swelling, most markedly in the proximal convoluted tubules where there is marked granularity, fragmentation and desquamation, the tubular lumina containing granular albuminous material but no casts.

Diagnosis: Marked cloudy swelling.

We are indebted to Mr. T. W. Thoburn, of the Third Year Class, for services rendered in connection with the work.



## THE SALICYLATES

### VII. FURTHER OBSERVATIONS ON ALBUMINURIA AND RENAL FUNCTIONAL CHANGES FOLLOWING THE ADMINISTRATION OF FULL THERAPEUTIC DOSES OF SALICYLATE \*

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In a previous communication<sup>1</sup> it was shown that the administration of salicylate in full therapeutic doses invariably causes the appearance of albumin, together with leukocytes and casts or cast-like bodies, in the urines of rheumatic and nonrheumatic individuals. The observations on renal function were inconclusive because of the variations and small differences in both the phenolsulphonephthalein excretion and nonprotein nitrogen of the blood before and after the administration of the drug. It was definitely shown, however, that the albuminuria is not of febrile, but of direct renal origin. The albuminuria, in some cases, is so severe that the presence of a nephritis is suggested. This was found to be the case in animals<sup>2</sup> (cats, dogs and a rabbit) receiving quantities of salicylate per kilo of body weight corresponding to the full therapeutic dose in human beings, in single and repeated doses, by mouth and subcutaneously. The pathologic changes in the kidney were interpreted as an acute tubular nephritis. An accumulation of non-protein and urea nitrogen in the bloods of a majority of the animals was also demonstrable. In other words, salicylate, in these animals, caused a diminution in renal functional efficiency, so far as these tests are concerned.

These findings encouraged us to extend our former observations, and arrive, if possible, at some final decision concerning the effects of salicylate on renal function in human beings. If it could be definitely shown that the drug injures the kidney, it should engender caution in its careless or promiscuous use as a remedy, particularly as to repeated administrations.

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1. Scott, R. W., and Hanzlik, P. J.: *Jour. Am. Med. Assn.*, 1916, **67**, 1838.

2. Hanzlik, P. J., and Karsner, H. T.: *THE ARCHIVES INT. MED.*, this issue, p. 1016.

TABLE 1.—CLINICAL DATA \*

Number and Patient	Diagnosis	Condition of Temper- ature	Fluid Intake	Toxic Dose of Salicyl,† Gm.	Diapho- resis	Urinary Changes						Adminis- tered Sodium Bicar- bonate Together With Salicyl, Gm.
						Albumin	White Blood Cor- puscles		Granular Casts or Castlike Bodies			
							Before Salicyl Medi- cation	During Salicyl Medi- cation	Before Salicyl Medi- cation	During Salicyl Medi- cation		
1 (P. F.)†	Rheumatic fever	Febrile	256 c.c. every hour for 12 hrs.; 256 c.c. every 2 hrs. thereafter	13.41	Moderate	—	+					
2 (J. M.)	Rheumatic fever	Febrile	170 c.c. every hour for 8 hrs.; 300 c.c. every 2 hrs. thereafter	12.81	Profuse	—	+	—	+	—	+	
3 (W. C.)	Rheumatic fever	Febrile	100 c.c. every hour for 9 hrs.; 200 c.c. every 2 hrs. thereafter	15.48	Profuse	+ tr.	+ inc.	+ oc.	+ inc.	+ oc.	+ inc.	
4 (J. H.)‡	Rheumatic fever	Febrile	220 c.c. every hour for 8 hrs.; 400 c.c. every 2 hrs. thereafter	14.45	Profuse	+	+ inc.	—	+	—	+	
5 (J. L.)‡	Rheumatic fever	Febrile	200 c.c. every hour for 8 hrs.; 400 c.c. every 2 hrs. thereafter	14.45	Very pro- fuse	+ tr.	+ inc.	+ oc.	+ inc.	—	+ oc.	
6 (F. D.)	Appendicitis T. B. kidney (?)	Afebrile	180 c.c. every hour for 7 hrs.; 300 c.c. every 2 hrs. thereafter	12.04	Mild	+ tr.	+ inc.	+ oc.	+ inc.	—	+	
7 (L. E.)	Tabes	Afebrile	150 c.c. every hour for 8 hrs.; 300 c.c. every 2 hrs. thereafter	13.76	Marked	+ tr.	+ inc.	+ oc.	+ inc.	—	+	
8 (H. M.)	Normal	Afebrile	150 c.c. every hour for 9 hrs.; 400 c.c. every 2 hrs. thereafter	15.48	Mild	—	+	+ oc.	+ inc.	—	+	
9 (A. P.)	Normal	Afebrile	100 c.c. every hour for 12 hrs.; 200 c.c. every 2 hrs. thereafter	14.96	Very mild	—	+	—	+	—	+	
10 (J. B.)	Normal	Afebrile	100 c.c. every hour for 11 hrs.; 200 c.c. every 2 hrs. thereafter	19.30	.....	+ tr.	+ inc.	—	+	—	+	
11 (S. V.)	Rheumatic fever	Febrile	100 c.c. every hour for 11 hrs.; 200 c.c. every 2 hrs. thereafter	18.33	Moderate	+ tr.	+ inc.	+ oc.	+ inc.	+	+ inc.	
12 (W. B.)	Rheumatic fever Old T. B. lesion of apex	..... Febrile	100 c.c. every hour for 5 hrs.; 200 c.c. every 2 hrs. thereafter	8.75	Very pro- fuse	+ tr.	+ inc.	—	+	—	+	
13 (D. T.)†	Normal	Afebrile	100 c.c. every hour for 7 hrs.; 200 c.c. every 2 hrs. thereafter	13.3	Mild	+ tr.	+ inc.	—	+	—	—	
14 (J. T.)‡	Normal	Afebrile	100 c.c. every hour for 6 hrs.	10.5	Mild	+ tr.	+ inc.	+ oc.	+ inc.	—	—	
15 (M. Z.)‡	Normal	Febrile	100 c.c. every hour for 6 hrs.	10.5	Marked	+ st.	+ inc.	+	+ inc.	+	+	
												14.0

16a (M. C.)‡	Lessened renal functional efficiency; hystoria	Afebrile	100 c.c. every hour for 6 hrs.; 200 c.c. every 2 hrs. thereafter	12.8	Mild	+ tr.	+ inc.	+ oc.	+ inc.	+ oc.	+ inc.	+ inc.
16b (M. C.)‡	Lessened renal functional efficiency; hystoria	Afebrile	100 c.c. every hour for 6 hrs.; 200 c.c. every 2 hrs. thereafter	11.4	Mild	+ tr.	+ inc.	+ oc.	+ inc.	+ oc.	+ inc.	+ inc.
16c (M. C.)‡	Lessened renal functional efficiency; hystoria	Afebrile	100 c.c. every hour for 7 hrs.; 200 c.c. every 2 hrs. thereafter	10.5	Mild	+	+ inc.	+ oc.	+ inc.	+ oc.	+ inc.	+ inc.
16d (M. C.)‡	Lessened renal functional efficiency; hystoria	Afebrile	100 c.c. every hour for 6 hrs.; 200 c.c. every 2 hrs. thereafter	9.36	Mild	+ tr.	+ inc.	+ oc.	+ inc.	+ oc.	+ inc.	+ inc.
17 (P. K.)	Chronic alcoholism	§	100 c.c. every hour for 7 hrs.; 200 c.c. every 2 hrs. thereafter	12.3	Mild	+	+ inc.	+ oc.	+ inc.	+ oc.	+ inc.	+ inc.
18 (F. S.)	Chronic alcoholism	Afebrile	100 c.c. every hour for 6 hrs.; 200 c.c. every 2 hrs. thereafter	10.8	Profuse	+	+ inc.	+	+ inc.	+	+ inc.	+ inc.
19 (Y. V.)	Tuberculosis	Very febrile	100 c.c. every hour for 6 hrs.; 200 c.c. every 2 hrs. thereafter	10.8	Moderate	+	+ inc.	+	+ inc.	+	+ inc.	+ inc.
20 (G. W.)	Chronic morphinism	Afebrile	100 c.c. every hour for 6 hrs.; 200 c.c. every 2 hrs. thereafter	10.8	Mild	+ tr.	+ inc.	+	+ inc.	+	+	+
21a (J. V.)	Rheumatic fever	Febrile	100 c.c. every hour for 6 hrs.; 200 c.c. every 2 hrs. thereafter	10.2	Profuse	+	+ inc.	+	+ inc.	+	+	+
21b (J. V.)	Normal	Afebrile	100 c.c. every hour for 5 hrs.; 200 c.c. every 2 hrs. thereafter	9.5	Mild	—	+ inc.	—	+	—	—	—
22 (E. L.)	Surg. (?)†	Afebrile	100 c.c. every hour for 5 hrs.; 200 c.c. every 2 hrs. thereafter	9.0	Mild	+ tr.	+ inc.	—	+	—	+	+
23 (E.)	Chronic rheumatism	Afebrile	100 c.c. every hour for 5 hrs.; 200 c.c. every 2 hrs. thereafter	9.5	Marked	+	+ inc.	+	+ inc.	+	+ inc.	+ inc.
24 (J. H.)	Endocarditis; cardiac decompensation	Afebrile	100 c.c. every hour for 5 hrs.; 200 c.c. every 2 hrs. thereafter	8.1	Mild	+	+ inc.	+	+ inc.	+	+ inc.	+ inc.
25 (H. V.)	Normal; photophobia	Afebrile	100 c.c. every hour for 5 hrs.; 200 c.c. every 2 hrs. thereafter	9.0	Mild	—	+	—	+	—	—	—
26 (R. H.)	Normal; ulcer of eye	Afebrile	100 c.c. every hour for 7 hrs.; 200 c.c. every 2 hrs. thereafter	12.6	Marked	+	+ inc.	+	+ inc.	+ oc.	+ inc.	+
27 (J. M.)	Normal; varicose ulcer	Afebrile	100 c.c. every hour for 7 hrs.; 200 c.c. every 2 hrs. thereafter	12.6	Mild	+	+ inc.	+	+	+ oc.	+	+
28 (T. S.)	Normal	Afebrile	100 c.c. every hour for 5 hrs.; 200 c.c. every 2 hrs. thereafter	9.0	Very mild	—	+	—	+	—	+	+ oc.

\* In this table the various signs and abbreviations have meanings as follows: plus (+) sign, present; minus (—) sign, absent; tr., trace; inc., increased; oc., occasional; st., strong.

† Salicyl was administered in form of sodium salicylate, expressed as salicylic acid.

‡ The albumuria in this individual was not studied quantitatively. In such cases, the urines were either utilized for other quantitative studies, or they had stood too long to give trustworthy results for albumin.

§ Partially febrile, temperature reaching a maximum of 99.8 F.

¶ A surgical condition, possibly renal; diagnosis incomplete.



It is the object of this paper to summarize the results of all of our observations on the effects on human renal functional efficiency. Observations on twelve additional individuals have been added to the sixteen previously reported, making a total, therefore, of twenty-eight different individuals, and thirty-two observations. A definite quantity (20 c.c.) of salicyl (usually of about 10 per cent. strength) in the form of sodium salicylate was administered, together with 80 c.c. of water, every hour until the subject complained of the well known symptoms of "toxicity." Then the administration was stopped, but the water was continued, usually 200 c.c. every two hours, until the excretion of salicyl in the urine ceased. That is, the fluid intake was maintained constant throughout as nearly as possible. Urine was collected every ten hours throughout the experiment and examined qualitatively (by the heat and acetic acid and ferrocyanid tests), and quantitatively (by the gravimetric method of Folin and Denis) for the presence of albumin, and microscopically for leukocytes, casts, etc., before and after the administration of the drug. Other tests of renal functional efficiency (before and after the administration of salicyl) were made as follows: The total nonprotein nitrogen of the blood was estimated according to the method of Folin and Denis,<sup>3</sup> using titration and distillation instead of the colorimetric procedure. The urea nitrogen content of the blood was estimated by the urease, aeration and colorimetric procedure.<sup>4</sup> The percentage excretion of phenolsulphonephthalein in two hours was studied in the usual way. The various clinical data pertaining to all the subjects are summarized in Table 1.

1. *Salicyl Causes Albuminuria and Aggravates a Pre-existing Albuminuria.*—In all the individuals of the present series, the administration of salicylate was followed by the presence of albumin, leukocytes and granular casts or cast-like bodies in their urines. This is, therefore, confirmative of our former observations. It is further confirmed that the albuminuria is not of febrile, but of direct renal origin, for it occurs in febrile, afebrile, rheumatic, nonrheumatic and normal persons.

Many of the individuals showed some degree of albuminuria before the drug was administered. It was, therefore, necessary to study the excretion quantitatively in order to ascertain definitely if this was increased by the salicylate. This was done by the gravimetric method of Folin and Denis.<sup>5</sup> The results from all the individuals studied (twenty-two) are presented in Table 2, and in Figure 1, which presents

3. Folin and Denis: Jour. Biol. Chem., 1912, **11**, 527.

4. We wish to express our thanks to Dr. Cyrus H. Fiske of the Biochemical Laboratory for suggesting the use of a glycerin extract of the jack bean for the urease.

5. Folin and Denis: Jour. Biol. Chem., 1914, **18**, 273.

TABLE 2.—EXCRETION OF ALBUMIN IN URINE OF INDIVIDUALS RECEIVING FULL THERAPEUTIC DOSES OF SALICYLATE

No.	Grams of Albumin Excreted in Each Ten-Hour Period									
	B. S.*	1	2	3	4	5	6	7	8	9
2	0.0	0.43	0.14	0.09	0.05	0.11	0.0	0.0	0.0	0.0
3	0.0	0.12	0.48	0.41	0.32	0.31	0.18	0.0	0.0	0.0
6	0.0	0.13	0.07	0.16	0.42	0.10	0.37	0.09	0.03	0.0
7	0.0	0.16	0.06	0.03	0.02	0.01	0.0	0.0	0.0	0.0
8	0.0	0.32	0.03	0.27	0.14	0.02	0.49	0.0	0.0	0.0
9	0.0	0.0	0.0	0.05	0.08	0.03	0.01	0.01	0.0	0.0
10	0.21	0.69	0.43	0.66	0.70	0.72	0.60	0.22	0.29	0.11
11	0.10	0.13	0.18	0.11	0.25	0.18	0.16	0.04	0.06	0.04
12	0.09	0.29	0.10	0.04	0.10	0.04	0.07	0.18	0.10	0.10
16b†	0.003	0.03	0.21	0.09	0.07	0.03	0.01	0.02	0.08	0.04
16c	0.05	0.01	0.10	0.08	0.05	0.15	0.08	0.02	0.06	0.006
16d	0.002	0.01	0.10	0.02	0.12	0.06	....	0.07	0.0	0.0
17†	0.04	0.20	0.05	0.24	0.06	0.10	0.06	0.02	0.03	0.14
18	0.11	0.16	0.13	0.05	0.08	0.03	0.09	0.05	0.03	0.04
19	0.06	0.19	0.27	0.47	0.42	0.25	0.31	0.38	0.003	0.13
20	0.01	0.38	0.28	0.02	0.006	0.02	0.07	0.05	0.05	0.02
21a	0.22	0.32	0.55	0.48	1.17	0.16	0.01	....	....	0.13
22	0.06	0.05	0.09	0.04	0.05	0.04	0.02	0.01	0.03	0.0
21b	0.0	0.04	0.13	0.06	0.02	0.0	0.0	0.0	0.0	0.0
23	0.02	0.07	0.08	0.03	0.01	0.09	0.11	0.0	0.26	0.08
24	0.0	0.06	0.31	0.22	0.23	0.24	0.05	0.08	....	0.04
25	0.0	0.06	0.09	0.12	0.14	0.08	0.06	0.02	0.0	0.0
26	0.05	0.28	0.12	0.12	0.46	1.98	0.58	0.21	0.0	0.0
27†	0.04	0.18	0.38	0.35	0.06	0.02	0.09	0.05	0.0	0.0
28†	0.0	0.09	0.03	0.04	0.02	0.07	0.05	0.02	0.0	0.0
Median	0.02	0.13	0.12	0.10	0.08	0.09	0.07	0.02	0.02	0.03

\* B. S. means "before salicyl."

† Received bicarbonate together with salicylate.

the course of the excretion by a curve of median values. This shows conclusively that a pre-existing albuminuria is aggravated by the administration of salicyl. It will be seen, also, that the albuminuria reaches its maximum at, or shortly after, "toxicity," and gradually tends to reach its previous level. As judged from the estimations of the salicyl content of these urines, the cessation of albumin excretion roughly coincides with the cessation of salicyl excretion. In a number of the individuals, however, the albuminuria tended to persist.

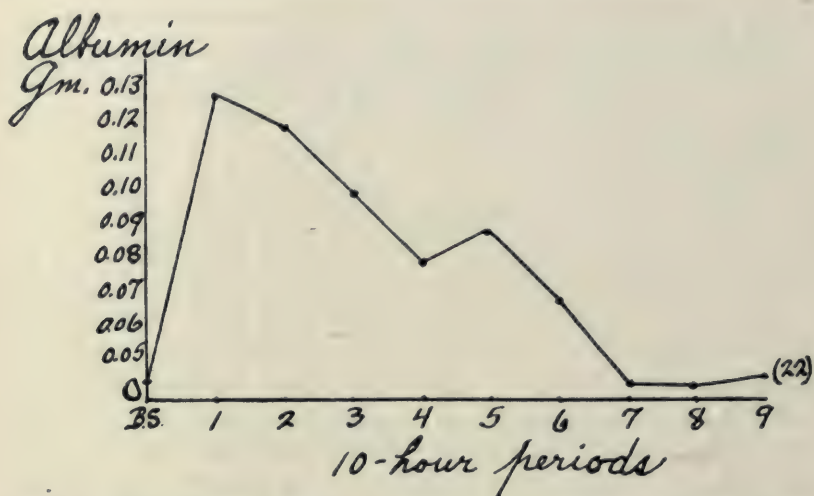


Fig. 1.—Excretion of albumin in urine in each ten-hour period following the administration of full therapeutic doses of salicylate. The curve represents median values; the number in parenthesis refers to number of different individuals; B. S. means "before salicyl."

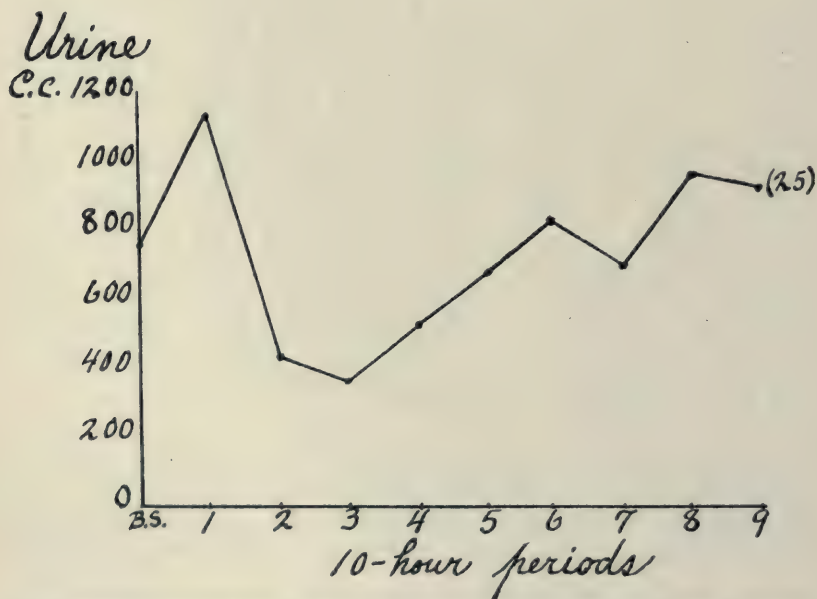


Fig. 2.—Excretion of urine in each ten-hour period following the administration of full therapeutic doses of salicylate. The curve represents median values; the number in parenthesis refers to number of different individuals; B. S. means "before salicyl."



TABLE 3.—EXCRETION OF URINE BY INDIVIDUALS RECEIVING FULL THERAPEUTIC DOSES OF SALICYLATE

No.	C.c. of Urine Excreted in Each Ten-Hour Period										
	B. S.*	1	2	3	4	5	6	7	8	9	10
1	....	1,720	1,123	703	428	404	1,060	1,858			
2	....	1,763	2,322	754	814	1,504	1,606	1,840	1,433		
3	....	2,001	446	320	274	343	325	638	943	864	791
4	....	2,153	1,094	1,307	1,884	1,586	1,586	2,080	1,642	2,144	2,073
5	....	1,074	714	579	634	400	662	464	1,491	1,191	928
6	....	3,239	469	1,315	899	1,071	978	708	670		
7	....	1,405	1,399	925	1,692	1,333	1,089	875	1,206	502	
8	....*	1,970	786	1,359	1,549	1,695	1,478	1,562	2,264		
9	....	810	550	625	230	360	185	690	755	1,027	
10	....	855	210	425	530	840	810	460	550	350	1,275
11	....	595	420	175	495	290	263	455	375	387	392
12	....	640	340	660	680	690	1,130	776	1,420	990	970
16b†	295	1,510	375	122	360	655	575	440	962	338	558
16c	670	1,230	290	127	115	290	119	275	760	110	105
16d	200	760	205	350	.....	1,315	505	.....	2,305	1,510	
17†	410	1,015	455	270	380	750	460	310	400	430	430
18	760	1,150	225	170	150	250	540	339	365	410	590
19	695	1,105	352	320	265	220	305	505	860	1,030	510
20	480	1,140	250	270	310	230	1,165	154	740	410	475
21a	370	950	340	320	340	235	.....	525	.....	315	250
21b	950	1,380	425	230	980	790	950	1,450	700	1,700	730
22	1,425	1,070	450	640	910	920	930	1,140	1,560	1,100	930
23	960	630	495	220	680	620	750	.....	580	980	
24	580	695	325	335	250	310	440	270	660	360	250
25	760	1,605	250	380	870	1,275	620	1,250	985	950	
26	1,160	1,995	605	395	240	1,075	1,035	1,340	1,125	1,100	
27†	965	1,300	705	330	1,085	510	1,450	1,030	1,160		
28†	1,250	905	850	400	875	1,170	1,260	620	1,280		
Median	760	1,145	448	365	530	673	810	690	953	907	

\* B. S. means "before salicyl."

† Received bicarbonate together with salicylate.

2. *Diuresis is Markedly Lessened.*—As indicated by the curve in Figure 2 and the data in Table 3 (from twenty-five different individuals), the excretion of urine is markedly diminished as a result of the administration of salicyl. The greatest depression is reached at, or shortly after, "toxicity," then the diuresis gradually increases and reaches practically its previous level about ninety hours after the

TABLE 4.—EXCRETION OF PHENOLSULPHONEPHTHALEIN IN THE URINES OF INDIVIDUALS RECEIVING FULL THERAPEUTIC DOSES OF SALICYLATE \*

Number	Per Cent. of Phenolsulphonephthalein Excreted in Two Hours					End of Experiment
	B. S.	A. T.	Daily observations After "Toxicity"			
			1	2	3	
6	60.0	55.0	60	..	..	60.0
7	55.0	60.0	55	..	..	60.0
8	70.0	60.0	65	..	..	70.0
16b†	30.0	35.0	..	..	..	35.0
17†	50.0	50.0	..	..	..	50.0
23	50.8	26.6	53	66	75	72.0
24	68.0	74.0	60	79	98‡	88.0
25	100.0	50.0	90	100	..	100.0
26	66.0	60.0	50	62	..	68.0
27†	89.0	75.0	76	94	..	90.0
28†	70.0	48.0	62	75	..	75.2
Median§	66.0	55.0	60	77	..	70.0
13	62.0	75.0				
14	70.0	50.0				
15	74.0	25.0				
16a	25.0	35.0				
16c	35.0	45.0				
16d	35.0	45.0				
18	60.0	60.0				
19	75.0	85.0				
20	75.0	75.0				
21	75.0	70.0				
22	75.0	80.0				
Median¶	70.0	60.0				

\* In this table, B. S. means "before salicyl"; A. T. means "at toxicity"; the end of the experiment was about eighty to ninety hours after administration.

† Received sodium bicarbonate together with the salicylate.

‡ Mean of 98, 83, 95, 100, 97, 88, 100, 100 and 92.5 per cent. of nine following days, respectively.

§ Used in the construction of the long curve in Figure 3.

¶ Used in the construction of the short curve in Figure 3.

administration of the drug. This diminution in water excretion is due, in part, to diaphoresis, which was usually marked, though variable, in all the subjects; and, in part, to retention; that is, edema, which we shall describe in a forthcoming contribution.

3. *Excretion of Phenolsulphonephthalein is Lessened.*—The curves in Figure 3 and the data (from nineteen different individuals and twenty-two observations) in Table 4 show that there is a diminution in

the two-hour excretion of phenolsulphonephthalein in the majority of persons after the administration of salicyl. This lasts for about twenty hours after administration is begun, and then gradually returns practically to its previous level. This diminution while relatively small, that is, about 10 per cent., is nevertheless definite, and quite constant in the majority of individuals.

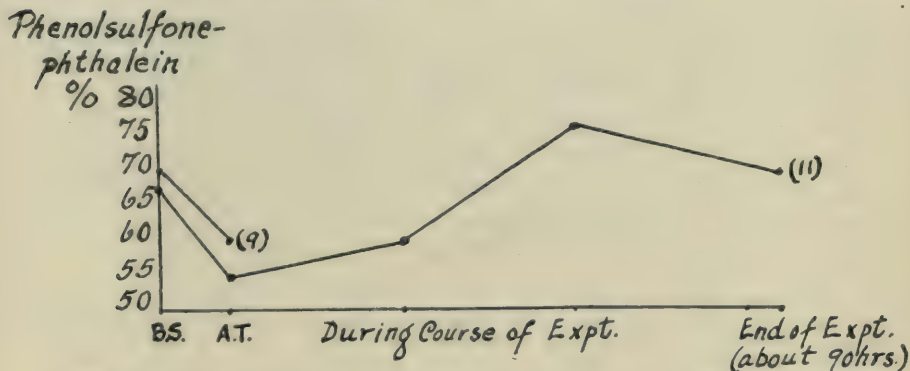


Fig. 3.—Excretion of phenolsulphonephthalein (two hours) in urine following the administration of full therapeutic doses of salicylate. B. S. means "before salicyl"; A. T. means "at toxicity," that is, about eight to ten hours after administration; daily observations were made "during the course of the experiment"; the numbers in parentheses refer to number of different individuals.

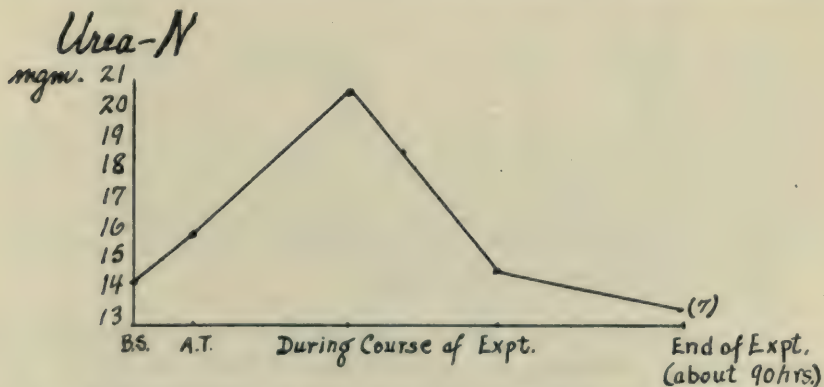


Fig. 4.—Accumulation of urea nitrogen of the blood (mg. in 100 c.c.) following the administration of full therapeutic doses of salicylate. B. S. means "before salicyl"; A. T. means "at toxicity," that is, at the end of eight to ten hours after administration; daily observations were made "during the course of the experiment"; the number in parenthesis refers to number of different individuals.

4. *Effect on the Nitrogenous Constituents of the Blood.*—Urea nitrogen: In the majority (seven) of persons in whom this was estimated, there was demonstrable a definite accumulation of urea nitrogen



in the blood. This begins to be evident about the time of "toxicity," reaches its maximum about twenty-four to thirty hours after administration, and returns to its previous level at the end of about eighty to ninety hours (three and one-third to three and three-quarter days). This is shown by the curve in Figure 4 and the data in Table 5. These seven persons represent the most complete study of the nitrogenous constituents of the blood of our series.

TABLE 5.—UREA NITROGEN CONTENT OF THE BLOOD OF INDIVIDUALS RECEIVING FULL THERAPEUTIC DOSES OF SALICYLATE \*

Number	Mg. Urea Nitrogen in 100 C.c. of Blood					
	B. S.	A. T.	Daily observations After "Toxicity"			End of Experiment
			1	2	3	
22	6.4	4.8	....	....	....	6.0
23	11.5	14.8	15.6	14.0	10.0	10.1
24	9.8	12.5	20.5	14.7	18.5†	13.5
25	16.0	17.0	14.5	16.5	....	14.9
26	17.2	16.0	20.6	19.0	....	16.0
27‡	14.3	17.6	21.4	13.4	....	13.4
28‡	19.5	21.6	20.6	....	....	14.0
Median§	14.3	16.0	20.6	14.7	....	13.5
17‡	28.0	22.0				
16d	9.1	8.3				
18	9.1	6.0				
19	16.3	3.0				
20	7.4	7.6				
21	15.4	16.2				

\* In this table, B. S. means "before salicyl"; A. T. means "at toxicity"; the end of the experiment was about eighty to ninety hours after administration.

† Mean of 12.3, 14.2, 12.1, 15.6, 13.5, 15.5, 12.5, and 13.5 for following eight days, respectively.

‡ Received bicarbonate together with salicylate.

§ Used for the construction of the curve in Figure 4.

In a number (six) of persons from whom it was impossible to obtain blood as frequently as was desirable, the results are not so conclusive, for the greatest accumulation of urea nitrogen would occur some time after the last specimen of blood was obtained. The blood was obtained only before the administration of the drug and at "toxicity." The results are presented in Table 5. It is seen there is a definite diminution of the urea nitrogen of the blood at "toxicity" in three (Patients 17, 18 and 19), and the content in the other patients was practically unchanged. It is quite likely that the important phase in the curve of accumulation of the urea nitrogen was missed in these experiments.

Nonprotein nitrogen: The results from nine different individuals are presented in Figure 5 and Table 6. These show a diminution in the nonprotein nitrogen content of the blood at "toxicity." This persists for a day or so, then gradually tends to return to its previous level at the end of the experiment, that is, about eighty to ninety hours after administration. The different individuals showed some variations in these changes.

As to the possible explanation of this diminution in the total nonprotein nitrogen content of the blood, it is suggested that the uric acid fraction, being affected by the salicyl, is perhaps chiefly responsible. In our series no attempt was made to estimate the uric acid content of the blood, but this has been conclusively shown by Denis<sup>6</sup> to be diminished in the blood after the administration of salicyl in large doses, and at the same time the excretion in the urine is increased. In addition, there are many observations by others showing definitely that salicyl

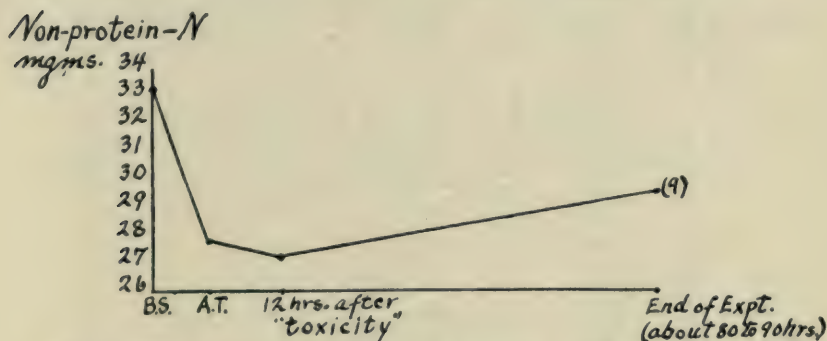


Fig. 5.—Total nonprotein nitrogen of the blood (mg. in 100 c.c.) after the administration of full therapeutic doses of salicylate. B. S. means "before salicyl"; A. T. means "at toxicity"; observations were also made twelve hours after "toxicity" and at the end of the experiment, that is, about eighty to ninety hours after administration; the number in parenthesis refers to number of different individuals.

increases the excretion of uric acid in the urine. The extent or degree of diminution in the total nonprotein nitrogen of the blood would to a certain extent depend on the content of the uric acid fraction before the administration of the drug, and in part on the functional state of the kidney. Because of this peculiar effect of the salicyl on the uric acid fraction, it appears that a study of the total nonprotein nitrogen content of the blood cannot serve as a reliable index of renal functional efficiency in individuals receiving large doses of the drug.

5. *Effect of Bicarbonate on the Albuminuria and Renal Functional Changes.*—It has been claimed (notably by Glaesgen<sup>7</sup> for human beings,

6. Denis: Jour. Pharm. and Exper. Therap., 1915, **7**, 255.

7. Glaesgen: München. med. Wehnschr., 1911, **58**, 1125.

and Frey<sup>8</sup> for rabbits) that the administration of bicarbonate together with salicylate, or rendering the urine alkaline during salicyl medication, tends to lessen or inhibit the albuminuria. Ehrmann,<sup>9</sup> who was not convinced that salicyl always caused albuminuria, claimed that alkalinity had no influence on the excretion of albumin in individuals in whom albuminuria occurred.

The results obtained by us do not support the claims that have been made for the beneficial effects of bicarbonate. Patients 13, 16b, 17, 27 and 28 received bicarbonate, together with salicylate, and the majority

TABLE 6.—TOTAL NONPROTEIN NITROGEN OF THE BLOOD OF INDIVIDUALS RECEIVING FULL THERAPEUTIC DOSES OF SALICYLATE \*

Number	Mg. of Nonprotein Nitrogen in 100 C.c. of Blood			
	B. S.	A. T.	12 Hours After "Toxicity"	End of Experiment
5	33.1	24.0	26.9	29.4
6	42.5	49.6	54.6	76.0
7	30.6	44.6	41.6	16.6
8	27.5	35.5	27.5	10.2
9	33.3	48.2	49.2	39.3
10	24.0	14.2	16.5	19.5
11	31.1	24.1	21.3	24.3
12	40.6	29.4	14.1	
16b†	35.1	20.9	....	43.3
16c	35.1	19.7	....	30.0
Median‡	33.2	27.8	27.2	29.4
12	66.3	63.1		
13	52.2	42.3		
14	52.2	52.5		
15	53.1	63.2		

\* In this table, B. S. means "before salicyl"; A. T. means "at toxicity"; end of experiment was about eighty to ninety hours after administration.

† Received bicarbonate together with salicylate.

‡ Used for the construction of the curve in Figure 5.

showed albuminuria, and of about the same degree and duration, diminution in water and phenolsulphonephthalein excretion, and accumulation of urea nitrogen in the blood. The results for these may be seen in Tables 1, 2, 3, 4 and 5. The urines of Patients 13, 16b, 17 and 27 remained alkaline throughout the experiment, and of Patient 28 only about forty hours after the administration was begun.

8. Frey: München. med. Wchnschr., 1905, **52**, 1326.

9. Ehrmann: München. med. Wchnschr., 1907, **54**, 2595.



Accordingly, bicarbonate has practically no demonstrable influence on the albuminuria and the changes in renal functional efficiency produced by the administration of salicyl in full therapeutic doses.

#### CONCLUSIONS

1. The administration of salicylate in full therapeutic doses invariably causes the appearance of albumin, white blood corpuscles and granular casts or cast-like bodies in the urines of normal, rheumatic, nonrheumatic, febrile and afebrile persons.

2. The albuminuria is not of febrile origin, but due directly to the drug.

3. A pre-existing albuminuria is aggravated by the administration of salicylate.

4. So far as renal functional efficiency is concerned, there is a diminution. This is indicated by, (1) lessened water excretion [taken in connection with (2) and (3)]; (2) diminished phenolsulphone-phthalein excretion, and (3) accumulation of urea nitrogen of the blood.

5. The administration of bicarbonate together with salicylate has practically no demonstrable influence on the albuminuria and renal functional changes produced by the salicyl.

# THE INFLUENCE OF NONSPECIFIC SUBSTANCES ON INFECTIONS \*

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The therapeutic measures used in the treatment of infections may be divided into two general classes: first, those specific substances which are supposed to destroy the infecting organism or neutralize its toxins, and second, those which aim to strengthen the natural processes that normally bring about recovery.

Of the specific substances which either destroy the infecting organism or render toxic products innocuous, we have specific antiserums and certain chemotherapeutic substances. Among the most effective antiserums may be mentioned diphtheria antitoxin, tetanus antitoxin, antimeningitis serum, and a serum for the treatment of one form of pneumonia. Many others have been prepared, but there is still some doubt as to their real value as therapeutic agents. Of the chemical substances which have a direct destructive action on the infecting organisms, quinin, salvarsan and emetin are probably the most important.

Bacterial vaccines, autogenous and stock, have been extensively used in the treatment of infections with the hope that they might cause an increased production of immune substances. Curative vaccine treatment rests primarily on the assumption that under certain conditions the production of specific antibodies can be increased by the injection of the bacteria causing the infection.

Originally, it was the belief that vaccines were indicated in those conditions in which the infecting organisms are localized and more or less encapsulated and thus unable to stimulate the body to produce a sufficient number of immune bodies to bring about recovery. According to these views it will be seen that the use of vaccines as a curative measure was restricted to localized infections.

It is a debatable question whether the practical results support the hypothesis on which specific vaccine therapy is based, and it will be difficult to explain by this hypothesis the results now being obtained in the treatment of acute systemic infections. In these conditions the infecting organisms are widely distributed through the body and should

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furnish the stimulus needed to call forth an abundant supply of antibodies.

When we compare the number of infectious conditions which a physician is called on to treat with the number of specific therapeutic substances at his command, it will be seen that in the great majority of instances he is compelled to rely on the use of agents which merely serve to strengthen and to sustain his patient. Recovery in these cases depends almost entirely on the gradually increasing concentration or activation of the normal protective agencies of the body, and the final results depends on the patient's ability to resist the infection until this stage is reached.

The work done in most of the experiments made to find curative agents for the various infections has evidently been based on the assumption that a substance must be found which will act directly on the infecting organism, or that will cause a mobilization of those specific immune bodies which we are accustomed to look on as the means nature uses to bring about recovery. And yet one finds a considerable accumulation of evidence, both from the laboratory and from the clinic, which definitely indicates that certain nonspecific and as yet ill-defined factors have a large share in bringing about recovery from disease. While the emphasis placed on certain phases of immunology and specificity, more especially the fruitful antigen-antibody conception of Ehrlich, has been of inestimable value, I am inclined to believe that it has resulted in the neglect of certain perfectly obvious lines of approach to medical problems.

#### SPECIFIC VACCINES

That specific vaccines are effective in the treatment of acute general infections is shown by numerous reports. In 1893, Fränkel<sup>1</sup> treated 57 typhoid fever patients by subcutaneous and intramuscular injections of typhoid bouillon cultures sterilized at 62 C., with excellent results. Petruschky,<sup>2</sup> in 1902, and von Peskarola and Quadrone,<sup>3</sup> in 1908, obtained similar results. During the Balkan war of 1913, Petrovitch,<sup>4</sup> inoculated subcutaneously 460 typhoid fever patients with typhoid vaccines, with a mortality of 2.9 per cent. Of 220 patients not treated in this manner, 12.8 per cent. died. Biedl<sup>5</sup> states that Wiel treated 14 children suffering from typhoid fever, without a single death, and that von Variot treated 69 patients by this method with excellent results. It must not be forgotten, however, that the mortality among children affected with typhoid fever is usually low.

1. Fränkel, E.: *Deutsch. med. Wchnschr.*, 1893, **19**, 985.

2. Petruschky, J.: *Deutsch. med. Wchnschr.*, 1902, **28**, 212.

3. Von Peskarola, B., and Quadrone, C.: *Ztschr. f. inn. Med.*, 1908, **29**, 989.

4. Petrovitch: Quoted by Biedl; see footnote 5.

5. Biedl, A.: *Prag. med. Wchnschr.*, 1915, **40**, 53.



Göer<sup>6</sup> treated successfully 9 cases of the same disease with a soluble albuminous product obtained from typhoid bacilli, and Krumbhaar and Richardson<sup>7</sup> observed favorable results in 77 cases treated subcutaneously with typhoid vaccines. These authors also found in the literature records of 1,800 cases of typhoid fever in which the patients were treated with subcutaneous injections of typhoid vaccines with favorable results.

During the past two or three years the methods of using vaccines in the treatment of acute general infections have undergone revolutionary changes. Ichikawa,<sup>8</sup> in particular, was instrumental in bringing about this change. This author gave intravenous injections of sensitized typhoid bacilli to 87 patients with typhoid fever. Immediate recovery, so far as fever and general toxic symptoms are concerned, followed the intravenous injection of one to two doses of the vaccines. Slight hemorrhages were observed in a few instances. Biedl<sup>5</sup> treated 21 patients with typhoid fever by the intravenous injection of typhoid vaccines prepared by various methods. Of these 21 cases, 2 patients died as a result of uncontrollable hemorrhages from the nose. Of the 19 remaining, 17 made immediate recoveries so far as temperature and toxic symptoms were concerned, one had a recurrence with an associated bronchopneumonia and died, and one patient had a slight recurrence which subsided immediately after a second dose. In discussing the explanation of these results he states that they may be due to the action of protein split products, aided by the mobilization of immune bodies, as was suggested by Ichikawa. Gay<sup>9</sup> treated typhoid fever patients with intravenous injections of sensitized typhoid bacilli, and states that the course of the disease was favorably influenced in 66 per cent. of the cases.

The above references show the results of the treatment of acute general infections by injections of specific vaccines. While the results may not have been entirely uniform in character, sufficient evidence is afforded to show that the older ideas concerning the principles on which vaccine therapy were based do not afford a satisfactory explanation.

#### NONSPECIFIC

Matthes,<sup>10</sup> early in the development of tuberculin therapy, demonstrated that to all intents and purposes the reaction considered specific for tuberculin could be produced when deuterio-albumose was used, and he showed that whatever difference did occur could be explained by

6. Göer, F.: *München. med. Wchnschr.*, 1915, **42**, 1312.

7. Krumbhaar, E., and Richardson, R.: *Am. Jour. Med. Sc.*, 1915, **149**, 406.

8. Ichikawa, S.: *Ztschr. f. Immunitätsforsch.*, 1915, **23**, 32.

9. Gay, F. P.: *THE ARCHIVES INT. MED.*, 1916, **17**, 301.

10. Matthes, M.: *Deutsch. Arch. f. klin. Med.*, 1894-1895, **54**, 39.

the fact that the tuberculin fraction contained certain toxic peptones in addition. Matthes, later, went even further, expressing the idea that fever in general was produced by protein split products, and he suggested the importance of proteolytic ferments in this connection, thus foreshadowing the work of Vaughan in this country and the later German workers in anaphylaxis.

Fränkel,<sup>1</sup> in 1893, was probably the first to demonstrate the value of typhoid vaccines in the treatment of typhoid fever, and his report was followed almost immediately by that of Rumpf,<sup>11</sup> who obtained similar favorable results with a vaccine composed of the bacillus pyocyaneus. The medical profession, however, could see no merit in a nonspecific method of treating infections; therefore the entire subject was dropped for a considerable period. In this country, too, the controversy occasioned by the Schäfer vaccines is pertinent. Here was a biologic product, which, according to the observations of many competent observers, did at times produce striking results in a variety of infectious diseases; whether or not it was a safe remedial measure, or whether it had defects, is not pertinent in this connection. The very fact that it did certain things at times should have led to a study of why such favorable changes were brought about. The fact that it was palpably nonspecific, however, was sufficient to warrant the stamp of disapproval by the medical profession.

Within the last three years, however, this view has been gradually changing, and it is significant to note that Wright,<sup>12</sup> than whom, of course, no one man has stood out more emphatically for specific therapy, recently made the following statement in this connection:

All of those who have had much experience with vaccines will have seen cases where therapeutic effects, lying quite outside the range of the particular vaccine employed, and therefore, as we thought, not quite creditable to science, have been obtained with vaccine therapy.

Schmidt<sup>13</sup> is among those who have observed that following vaccine therapy of any kind the body becomes resistant to a variety of commoner infections. He also called attention to the relatively low "infection index," as he termed it, which is present in carcinoma and pregnancy, and refers to Rokytansky's ideas on the antagonism between carcinoma and tuberculosis.

Von Wagner<sup>14</sup> utilized a similar nonspecific method when he obtained favorable results in patients with progressive paralysis treated with tuberculin. He had observed, as had others, that intercurrent infections frequently cause a remission of symptoms in this disease

11. Rumpf, T.: *Deutsch. med. Wchnschr.*, 1893, **19**, 987.

12. Wright, A.: *Brit. Med. Jour.*, 1915, **1**, 625.

13. Schmidt, R.: *Med. Klin.*, 1910, **6**, 1690.

14. Von Wagner, J.: *Wien. med. Wchnschr.*, 1909, **59**, 2124.



which sometimes last for a long period. This led him to experiment first with tuberculin alone, and later with a combined tuberculin-antiluetic treatment. He believed that he got better results with the combined treatment than with the antiluetic treatment alone. Pilcz<sup>15</sup> observed similar good results with tuberculin, while Donath,<sup>16</sup> noting the same influence of intercurrent infections in paretics, suggests the formation of abscesses in these patients by injecting turpentine into the subcutaneous tissues.

It is not intended here to recommend the use of tuberculin in the treatment of paretics, because it hardly seems warranted, but to indicate the same general phenomenon of a therapeutic effect from a non-specific method.

It is in the domain of coagulation disturbances that therapy of this nature has received particular attention in the past. The beneficial effects of subcutaneous serum injections in hemophilia is well recognized, although here too the exact mechanism is unknown. Originally, the slight leukocytosis was regarded as the potent factor, but for this there is no proof. Results have been obtained with homologous and heterologous serums, and with whole blood; but decisive results depend considerably on the dosage, and marked fluctuations in the coagulation time frequently occur following these injections. That such injections do not alter the disease process through supplying directly some deficiency, but rather through stimulating the elaboration of some substance before lacking, seems to be the conclusion reached by recent workers. This would agree with the stimulating effects observed by Esch, Busse and Weber following the injection of serum and whole blood subcutaneously in tuberculosis and in pernicious anemia.

The dermatologists have had similar results within the past three years in a variety of diseases, including some cases of psoriasis which have proved refractory to other methods of treatment. Recently Engman and McGarry<sup>17</sup> report favorable results in the treatment of lupus erythematosus with intravenous injections of typhoid bacilli.

The therapy of typhoid fever has so far been the chief avenue of approach to the problem. A number of vaccines have been elaborated and used subcutaneously during recent years for therapeutic purposes, and the results have been, in general, very encouraging. It was not, however, until such vaccines were used intravenously that the striking pictures of complete abortion of the disease were obtained.

Ichikawa,<sup>8</sup> who was among the first to use the intravenous method of administering vaccines in typhoid fever, observed that the results in

15. Pilcz, A.: *Wien. med. Wchnschr.*, 1907, **57**, 1462.

16. Donath, J.: *Wien. med. Wchnschr.*, 1916, **67**, 1741.

17. Engman, M., and McGarry, R.: *Jour. Am. Med. Assn.*, 1916, **67**, 1741.



patients suffering from paratyphoid fever who had been treated with typhoid vaccines were similar in every way to those noted in typhoid fever. Kraus<sup>18</sup> obtained similar results in the treatment of typhoid fever with the intravenous injections of vaccines composed of colon bacilli. He also treated eight patients with puerperal sepsis with excellent results. Kraus was so impressed with this form of treatment that he suggests its use with scarlet fever, plague, septicemia, etc. In addition to vaccines prepared with cholera bacilli, typhoid bacilli and paratyphoid bacilli, Lüdke<sup>19</sup> also used deuterio-albumose. He treated twenty-three typhoid fever patients, and of these, nineteen cases were favorably influenced. There was one death in this series. Reibmayr<sup>20</sup> obtained similar results with colon and cholera vaccines.

Hiss and Zinsser<sup>21</sup> were among the first to make use of nonspecific substances in the treatment of acute infections. They used extracts of rabbits' leukocytes in the treatment of epidemic cerebrospinal meningitis, in pneumonia, and in staphylococcus infections, and believe that the course of these diseases was favorably influenced. Floyd and Lucas<sup>22</sup> used the leukocytic extracts in the treatment of forty-one cases of pneumonia, and report that the mortality in their series was about half that obtained in the same institution with other forms of treatment.

Miller and Lusk<sup>23</sup> during the summer of 1916 treated a series of patients with typhoid fever with intravenous injections of typhoid bacilli and with secondary proteoses. In this series, 20 per cent. terminated by crisis, and 20 per cent. by rapid lysis. They also treated a series of chronic, subacute and acute cases of arthritis with these preparations with excellent results, as relief was afforded in the majority of cases. The authors do not state the number of patients treated by each method, but conclude that both gave the same results. The same authors<sup>24</sup> recently reported a second series consisting of eighty-five patients with arthritis treated with typhoid vaccines. Forty-five of these cases were acute, four being gonorrheal. In twenty-nine of the acute cases the patients recovered promptly after one to four injections, eight showed marked improvement, six moderate improvement, and two were unimproved. Nine of these patients had recurrences. There were twelve subacute cases, ten of which cleared up completely within three to five days, and two patients were greatly improved. There were two recurrences in this group, but the patients made complete recoveries

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18. Kraus, R.: *Wien. klin. Wchnschr.*, 1915, **38**, 29.

19. Lüdke, H.: *München. med. Wchnschr.*, 1915, **62**, 321.

20. Reibmayr, H.: *München. med. Wchnschr.*, 1915, **62**, 610.

21. Hiss and Zinsser, H.: *Jour. Med. Research*, 1908, **19**, 321.

22. Floyd, C., and Lucas, W.: *Jour. Med. Research*, 1909, **21**, 223.

23. Miller, J., and Lusk, F.: *Jour. Am. Med. Assn.*, 1916, **66**, 1756.

24. Lusk, F., and Miller, J.: *Jour. Am. Med. Assn.*, 1916, **67**, 2010.

on further treatment. Nineteen chronic patients were treated, ten of which showed definite improvement.

Culver<sup>25</sup> reports a series of gonorrheal complications, especially arthritis, epididymitis and acute prostatitis, in which the patients were treated with a variety of vaccines composed of gonococci, meningococci, colon bacilli, and with secondary proteoses. Twenty-eight of the thirty-one arthritic patients were either completely cured or manifested a decided improvement. The twelve patients with acute epididymitis presented complete freedom from pain after the first injection.

Ziembowski<sup>26</sup> has recently reported on the results obtained in the treatment of 100 patients with intramuscular injections of 5 c.c. of boiled milk. He states that excellent results were obtained by this means in the treatment of septic war wounds, in erysipelas, in tuberculous bone and joint diseases, and in three cases of actinomycosis.

Matthers, in a personal communication, states that he has used various types of vaccines and pure proteins in the treatment of typhoid fever, lobar pneumonia, scarlet fever and acute arthritis. He infers from his experiments that the therapeutic results obtained in erysipelas and lobar pneumonia do not justify the use of this plan of treatment. In the other diseases mentioned his results correspond favorably with those reported by other investigators.

Manier, Petersen and I have treated thirteen cases of arthritis, of which three were acute, three subacute and seven chronic. Of the acute cases, two were definite cases of acute rheumatic fever. One of these patients, a child of 12 years, with multiple swollen, tender joints, fever, etc., cleared up entirely within twenty-four hours after a single infection of secondary proteoses, while the other, an adult female with multiple joint involvement, elevation of temperature, etc., was entirely relieved after eight injections, except for some residual pain in one shoulder. The third acute case, with a gonorrheal joint, received four injections, with only temporary subjective relief and with but slight objective change in the joint. In the three subacute cases the patients received an average of five injections, with complete relief of all symptoms and a return of the involved joints to normal in every respect.

In the seven cases of chronic arthritis there were varying degrees of disability, from slight stiffness in the milder cases up to complete ankylosis of the majority of the joints of the body in the most severe cases. The injections given the patients varied in number from two to eighteen. The results were, complete relief in three, marked improvement in three and no noticeable change in one.

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25. Culver, H. B.: Unpublished address before Chicago Institute of Medicine, Nov. 7, 1916.

26. Ziembowski, S. V.: *Med. Klin.*, 1916, **12**, 1174.



Four patients with gonorrheal epididymitis with acute swelling and tenderness of the epididymis, urethral discharge, etc., were treated. In every case of this series there was immediate improvement following the first injection, the epididymis becoming smaller and distinctly less tender. In each patient there was complete relief, as evidenced by the return of the epididymis to its normal size and consistency, together with the disappearance of the urethral discharge and of pus from the urine.

Two patients with erysipelas were treated. One was first seen on the second day of the disease, at which time the condition involved almost the entire face, and, in addition, the mucous membrane of the mouth and of the pharynx. The patient was markedly toxic, with a temperature ranging from 100 to 103 F., and a pulse rate of 140. He was given two doses of a secondary proteose solution on successive days, following which his temperature and pulse rate promptly settled to normal, and his local condition, both on the face and in the mouth, cleared up promptly. The second patient was also seen on the second day of the disease, and while he was not so seriously ill, the symptoms did not clear up until after four injections.

The amount of secondary proteoses given by us depends on the apparent toxicity of the patient. We usually begin with 0.25 c.c. of a 1 per cent. solution and increase the dose according to the reaction obtained. When it is remembered that Lüdke,<sup>19</sup> Miller and Lusk,<sup>23</sup> and Culver<sup>25</sup> gave as high as 2 c.c. of a 4 per cent. solution, it will be seen that the amounts given by us are small. Subsequent experience, however, may demonstrate that the larger doses are advisable despite the more severe reactions, and particularly in arthritic cases, where the reactions appear to be associated with little or no danger. Our purpose has been to obtain some increase in temperature with a slight chill, and this reaction we can usually obtain at the first injection with 0.25 c.c. of a 1 per cent. solution. Injections were given in most instances every day. As individuals vary in their reactions, we believe it better to begin with the smaller dose and thus establish the tolerance of each patient before pushing the treatment. In no instance were there any alarming symptoms.

It is almost impossible to determine beforehand the degree of reaction which will follow these intravenous injections. Some authors believe that it depends on the severity of the disease, while others consider the concentration of immune bodies in the circulating blood more important. From a general survey of the work done, however, it appears that there is some other factor, as yet unknown, which plays an important part in determining the severity of the reaction.



Apart from the action of the vaccines, the protein split products, and the effect of both homologous and heterologous serums, observations are recorded by Smithlen,<sup>27</sup> Müller and Weiss,<sup>28</sup> and Saxl, Bruck and Kiralihyda<sup>29</sup> on the effect of intramuscular injections of milk, by Mitlander<sup>30</sup> on salt solution, and by numerous observers on the effects of dextrose solution, colloidal metals, distilled water, etc. It seems probable that the reaction and beneficial effects observed from these substances is based on a similar mechanism in all cases.

#### REACTION

Immediately following an injection by this method of treatment there is usually a reaction which is sometimes severe. As a rule, there is a chill from one-half to one hour following the injection, and this may last fifteen to forty-five minutes. With the chill there is an increase in temperature of from 1 to 4 F., followed several hours later by a progressive fall. Associated with the drop in temperature there is general relaxation, profuse perspiration and a rapid subjective and objective improvement. The pulse may or may not be increased in frequency. The blood pressure in our own cases was not altered. Some authors report that many of their patients had headache, nausea, etc., and again others state that the symptoms following the injections are not sufficient to cause serious discomfort. These differences may depend on the dosage used.

Following the injection the leukocytes are decreased in number, at times as low as 2,000 per cubic millimeter. This leukopenia is followed by a gradually developing leukocytosis which usually reaches its maximum in from five to seven hours. The leukocyte count has usually returned to normal within twenty-four to thirty hours.

Immediately following the injection in acute infections, such as typhoid fever, there may be a permanent return to normal temperature — termination by crisis; the temperature and general conditions may improve more slowly — termination by lysis; or, all the symptoms may return and the disease progress as usual, uninfluenced in any manner, though usually it pursues a milder course. The temperature frequently drops to subnormal and remains so for several days in those cases which terminate by crisis.

#### COMPLICATIONS AND CONTRAINDICATIONS

The only serious results that have followed the use of this form of treatment occurred in typhoid fever patients, but as a large majority

27. Smithlen, F.: *Wien. klin. Wchnschr.*, 1916, **29**, 53.

28. Müller, R., and Weiss, A.: *Wien. klin. Wchnschr.*, 1916, **29**, 249.

29. Saxl, Bruck and Kiralihyda: *München. med. Wchnschr.*, 1916, **63**, 511.

30. Mitlander: *Budapest Letter, Jour. Am. Med. Assn.*, 1916, **66**, 1321.

of the cases of acute general infections treated were in patients with typhoid fever, it is important to learn more concerning the danger attached to treating this class of patients with nonspecific substances.

Hemorrhage is the most serious complication reported in typhoid fever. Ichikawa<sup>8</sup> who used sensitized typhoid vaccines in eighty-seven cases, observed hemorrhages in a few patients one to three days after inoculation, but the frequency with which these were observed was less than that noted among the uninoculated. R. Schmidt<sup>13</sup> advises against its use in patients who have already had hemorrhages, or who give histories of having been bleeders. According to this author, bronchial and pulmonary complications also contraindicate its use. Biedl<sup>5</sup> treated eighty-four typhoid fever patients with typhoid vaccines, and two of these died from uncontrollable hemorrhages from the nose. He also believes that previous hemorrhages contraindicate this form of treatment.

Typhoid fever appears to be the only disease in which hemorrhages have been observed following this method of treatment. Kraus<sup>18</sup> does not mention it as occurring in his cases of general sepsis; it was apparently not observed in any of the paratyphoid cases, and Miller and Lusk<sup>23</sup> do not mention its occurrence in their series of more than one hundred arthritic cases.

According to R. Schmidt,<sup>31</sup> protein substances injected intravenously or subcutaneously tend to decrease the coagulation time, and it is well known that certain of the lower protein cleavage products also inhibit coagulation. On the other hand, the subcutaneous and intravenous injection of homologous and heterologous serums has been a favorite procedure for some time in the treatment of hemophilia.

Ichikawa,<sup>8</sup> Miller and Lusk,<sup>23</sup> and others, advise against this method of treatment in patients with organic heart disease, and Ichikawa warns against its use in pregnancy. In the opinion of Lusk and Miller its use is also contraindicated in hypertension.

The severity of the reaction is an important factor in determining whether this form of treatment should be used in any particular instance. When we consider that the strength of the vaccines of specific and nonspecific nature which have been used in the treatment of typhoid fever varied from 100,000,000 to 4,000,000,000 bacteria to the cubic centimeter, it will be seen that the danger to the patient is not so great as the severe reactions would indicate.

And now, how are we to explain the action of these nonspecific substances? Can it be explained according to our present ideas of nature's method of bringing about recovery from infection? I believe it is doubtful if our present theories on immunity will enable us to

31. Schmidt, R.: *Med. Klin.*, 1916, **12**, 171.

explain the action of these nonspecific substances. It might be well, however, to take up in greater detail some of the explanations which have been advanced.

#### SELECTIVE STIMULATION

It is now the general belief that the hematopoietic organs are the chief source of antibodies, and not the tissue cells in general. As a corollary of this idea concerning the source of antibodies, it would be reasonable to suppose that any disturbance of the hematopoietic system might alter the antibody formation.

In view of these facts, it is possible that the various agents may act as stimulants of the hematopoietic tissue, thus suddenly flooding the body with immune substances, thereby overcoming the infection. According to Wright, vaccine injections were supposed to be followed by a negative phase, at least so far as the opsonic power was concerned. Contrary to this generally accepted view, Bull<sup>32</sup> has recently shown that this does not hold true following the intravenous injection of a typhoid vaccine in immunized rabbits. Bull noticed that the antibodies were not diminished; on the contrary, they were rapidly increased following the injection. If this is the mechanism involved, it is important to bear in mind that the stimulus itself is not a specific factor, but that the hematopoietic system has been attuned to respond to a non-specific stimulus with the production of a specific substance.

Various investigators have stated that the results obtained with these nonspecific substances are due to the mobilization of antibodies. Thus Müller and Weiss<sup>28</sup> thought this was the explanation of the results which they obtained in treating the complications of gonorrhea with gonorrheal vaccines, but serologic tests failed to confirm this view. Ichikawa<sup>8</sup> attributed his results to the mobilization of antibodies. Kraus<sup>18</sup> believed at first that the phenomenon was very similar to anaphylactic shock, but concludes that the lack of specificity contradicts this view. For the same reason he believes that the results obtained are not due to a mobilization of immune bodies. Lüdke<sup>19</sup> found that the agglutination value of the serum of typhoid patients treated with proteoses was not changed. Reibmayr,<sup>20</sup> also, found no changes in the agglutinins following the injections of typhoid vaccines.

In this respect the observations of Moreschi<sup>33</sup> are interesting. Moreschi noted the persistent absence of agglutinins in leukemic patients who suffered from superimposed typhoid and paratyphoid infections. The patient with typhoid fever recovered, while the one with the paratyphoid infection died. Immune bodies could not be

32. Bull, C.: *Jour. Exper. Med.*, 1916, **23**, 419.

33. Moreschi, C.: *Ztschr. f. Immunitätsforsch.*, 1914, **21**, 410.



demonstrated in either case. The recovery of the typhoid patient indicates that agglutinins may not be essential.

Experiments which we conducted last spring caused us to believe that the results obtained in the use of nonspecific substances in the treatment of infections, were due to the mobilization of immune bodies. Dunklin, who was working with us, found a marked increase in antibodies following the intravenous injection of proteoses in immunized animals. A similar increase in agglutinins was found in two cases of typhoid fever in which the patients had been treated with the same preparation of proteoses. We have not had the opportunity to make further tests in typhoid fever patients, but repeated experiments made during the past few months have failed to show an increase of antibodies in immunized animals following similar intravenous injections. The arthritic cases do not afford opportunities for this character of investigations, therefore we have been limited in our work to animal experiments. The observations of others, however, appear to show that the mobilization of antibodies must play a minor rôle in recovery from infection following the use of nonspecific substances.

#### HYPERPYREXIA

It is a common clinical experience that in some diseases, among them subacute joint diseases, neuralgia, diabetes, pernicious anemia, certain dermatoses, sarcoma, etc., distinct beneficial results follow at times on some intercurrent febrile condition. May it not be, then, that these nonspecific substances influence the course of the disease by producing a high temperature?

Rolly and Meltzer,<sup>34</sup> Lüdke,<sup>35</sup> and other investigators, have reached the conclusion that high temperature (from 40 to 42 C.), artificially produced, has a favorable influence on an established infection. Heated animals were distinctly more resistant to daily injections of small quantities of bacteria, but no difference was noted when single large doses were given. They also found that agglutinins and bacteriolytic substances are produced more abundantly in animals which are kept overheated.

In his discussion of the influence of high temperatures on infection, Lüdke<sup>35</sup> suggests, first, the possibility of the infecting organism being killed by the heat, and second, that a more rapid and firmer combination of the antigen and immune bodies is caused at high temperatures.

Culver<sup>25</sup> describes an instance in which a patient suffering from both acute gonorrheal urethritis and malaria, made a complete recovery from the urethritis following chills and fever lasting four days. He

34. Fr. Rolly and Meltzer: *Deutsch. Arch. f. klin. Med.*, 1908, **94**, 335.

35. Lüdke, H.: *Ergebn. d. inn. Med. u. Kinderh.*, 1909, **4**, 493.

also states that one rarely sees a gonorrheal infection coexisting with fever producing diseases like pneumonia, typhoid fever and malaria. It is well known that gonococci are particularly susceptible to high temperatures, and therefore this factor may be of importance in gonococcal infections, but it is doubtful if similar importance can be ascribed to high temperatures in infections due to such organisms as streptococci, typhoid bacilli, etc.

Inasmuch as a very sharp febrile reaction almost invariably follows the intravenous injection of specific and nonspecific substances, the importance of this phase of the subject cannot be overlooked. In one of our cases we observed a reaction temperature of 107 F. within thirty minutes after an intravenous proteose injection. This high temperature was unaccompanied by a change in the pulse rate of any moment, or other untoward symptoms.

#### LEUKOCYTOSIS

The importance of the leukocytic reaction has been emphasized by various authors. Gay<sup>36</sup> and his associates believe that recovery in typhoid fever following the intravenous injection of a modified typhoid vaccine is due to a specific leukocytosis. More recently, however, McWilliams<sup>37</sup> has observed that this hyperleukocytosis is apparently not specific to the degree indicated by the work of Gay and Claypole, and that even normal rabbits respond with a marked leukocytosis to the intravenous injection of typhoid vaccine. This coincides with our experience. We must keep in mind, too, the fact that in typhoid fever, particularly, the normal course of recovery is not marked by a leukocytosis; on this ground alone we might be justified in seeking the influence of some other factor or factors in the recovery which follows intravenous therapy.

On the other hand, Lüdke<sup>19</sup> states that there was no leukocytosis in his series of typhoid fever patients who were treated with albumoses, and Reibmayr<sup>20</sup> makes a similar statement for his series of patients treated with typhoid vaccines. Most of those who believe that the leukocytosis is an important factor in recovery think chiefly of phagocytosis; other possibilities, however, must be considered.

Hiss and Zinsser<sup>21</sup> say that immunity is probably in a large degree cellular in character, not only in the sense of phagocytosis, but also in the neutralization of toxins. They conducted a series of experiments with this idea in mind, using the leukocytes of rabbits, and came to the conclusion that "leukocytic extracts have a distinct modifying and curative action on infections." They believe that the results

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36. Gay, F., and Claypole, E.: *THE ARCHIVES INT. MED.*, 1914, **14**, 662.

37. McWilliams, H.: *Jour. Immunol.*, 1916, **1**, 259.

are due to the neutralization of endotoxins. Opie<sup>38</sup> found that leukocytes injected into the pleural cavities of dogs in which a tuberculous pleurisy had previously been produced, tended to inhibit the development of the process, as those animals which received the leukocytes lived longer than the controls. Recently, Bail<sup>39</sup> has advanced evidence that supports this theory. He found that a strong anticholera serum would not neutralize the endotoxin obtained from cholera bacilli, but that the toxin was destroyed if it was first incubated with a fresh emulsion of leukocytes and the antiserum then added. In these experiments the leukocytes were removed by centrifuging before the serum was added. Control experiments demonstrated that the leukocytic emulsion alone did not destroy the toxin. Heating the leukocyte emulsion thirty minutes at 58 C. destroyed its antitoxic action.

Bull and I<sup>40</sup> showed that leukoprotease will destroy the toxic extracts of typhoid bacilli and meningococci, and it is not improbable that a similar explanation will apply to the results obtained by Bail.

It will be seen, then, that in considering the part leukocytosis may play in the recovery from disease, we must consider other factors in addition to phagocytosis.

#### MOBILIZATION OF FERMENTS

Petersen and I have already pointed out that in experimental animals intravenous injection of bacteria,<sup>41</sup> kaolin,<sup>42</sup> protein split products<sup>43</sup> and trypsin<sup>44</sup> is almost invariably followed by more or less marked mobilization of serum protease and usually of esterase.

Similar reactions occur in patients following the intravenous injection of vaccines and proteoses, but not to the same degree nor with the same regularity as in animals. In considering the possible effects of such a mobilization of ferments, both protease and esterase, we must keep in mind the fact that a variety of reactions may occur. The serum protease, as other tryptic ferments, is without effect on bacteria;<sup>45</sup> but if we consider the source of the intoxication which occurs in the diseased organism as primarily due to protein split products derived from the bacteria, then such a mobilization of protease may be of considerable importance in the process of detoxication, as the toxic fragments are hydrolyzed to lower and nontoxic forms. Petersen,

38. Opie, E.: *Jour. Exper. Med.*, 1908, **10**, 419.

39. Bail, O.: *Ztschr. f. Immunitätsforsch.*, 1916, **25**, 248.

40. Jobling, J. W., and Bull, C.: *Jour. Exper. Med.*, 1913, **17**, 453.

41. Jobling, J. W., and Petersen, W.: *Jour. Exper. Med.*, 1915, **22**, 590.

42. Jobling, J. W., and Petersen, W.: *Jour. Exper. Med.*, 1915, **22**, 597.

43. Jobling, J. W., and Petersen, W.: *Jour. Exper. Med.*, 1915, **22**, 603.

44. Jobling, J. W., and Petersen, W.: *Jour. Exper. Med.*, 1915, **22**, 141.

45. Jobling, J. W., and Petersen, W.: *Jour. Exper. Med.*, 1914, **16**, 321.



Eggstein and I<sup>46</sup> have discussed this possibility in detail in its relation to pneumonia.

As a result of this action of the proteolytic ferments the diseased organism would rid itself for the time being of the toxic substances in the circulating blood, although the disease process itself, and the infecting organisms, would possibly continue in existence, causing further injury. This would seem to be the explanation of the clinical picture obtained after intravenous therapy in those instances in which only an incomplete or transitory effect results. In the majority of these cases the patient presents a considerable improvement, both subjectively and objectively, on the day or days following the injection, despite the fact that the temperature may recur or even continue uninterrupted. This hypothesis will not, however, explain the continued wellbeing of the patients treated in the early stages of diseases such as typhoid fever. It is difficult to understand this unless it is due to cellular resistance acquired as a result of the injections.

The influence of the mobilized ester-splitting ferments is as yet obscure. In the ultimate analysis, of course, we must turn to ferments of this nature in order to dispose of the invading organism, because it is more than probable that the actual surface of the bacterium consists largely of lipoids, or intimate lipoprotein combinations, for the destruction of which esterase-splitting ferments are probably essential.

Citron and Reicher,<sup>47</sup> Peritz,<sup>48</sup> and others, state that the serum esterase is increased in patients who suffer from infections due to lipid rich organisms such as tubercle bacilli and lepra bacilli. They believe that a high esterase titer in these diseases indicates increased resistance on the part of the host.

#### ANTIFERMENTS

When discussing the increased resistance to infections said to be present in some conditions, among them carcinoma, pregnancy, and as a result of vaccine therapy, I called attention to the fact that during such states a relatively high antiferment index is a well recognized accompaniment. The question arises whether this is a mere coincidence, or whether there is some causal connection between the antiferment increase and the increased resistance to infection.

Petersen and I<sup>49</sup> have previously shown that the antiferment power of the serum depends on the amount of unsaturated lipoids present in a highly dispersed state in the serum. Consequently, any factor which

46. Jobling, J. W., Petersen, W., and Eggstein, A.: *Jour. Exper. Med.*, 1915, **22**, 568.

47. Citron, J., and Reicher, K.: *Berl. klin. Wchnschr.*, 1908, **45**, 1398.

48. Peritz, G.: *Deutsch. med. Wchnschr.*, 1910, **36**, 483.

49. Jobling, J. W., and Petersen, W.: *Jour. Exper. Med.*, 1914, **19**, 459.

will tend to increase these lipoids, either by increasing the supply or by decreasing the utilization, will increase the antiferment titer, while conversely, any influence decreasing these lipoids, their dispersion or their unsaturation, will tend to decrease the titer.

Wright<sup>50</sup> worked with saprophytic and serosaprophytic organisms, and noted that the addition of antiferment to the culture medium completely checked the growth of the serosaprophytic bacteria. He also found that even the saprophytic bacteria grew less luxuriantly when no proteolytic ferment was added. The direct influence of the antiferment cannot be as simple as Wright would assume, as Rettger, Berman and Sturges<sup>51</sup> showed that the ordinary pathogenic organisms do not derive their protein requirements from native or even partly hydrolyzed proteins, but solely from the lowest split products. The antiferment inhibits only the action of tryptic and not the peptolytic and ereptic ferments. Of course, when we are dealing with a definite tryptogenic organism it becomes apparent that an increase in antiferment would offer an increased factor of resistance against its growth.

The immediate effect of the vaccine and proteose injections is not an increase, but a distinct decrease, in the antiferment titer for a short period of time, followed later by a rise. The exact cause of these changes in the antiferment index remains undetermined.

#### PHYSICAL CHANGES IN SERUM

We have recently found that there are distinct changes in the viscosity of the serum of animals undergoing immunization, and that similar changes occur in anaphylactic shock. This alteration may be of more than theoretical interest in our interpretation of the results obtained by the intravenous injections of nonspecific substances.

Weil<sup>52</sup> and others have recently shown that antigen and antibody may coexist in the blood of the living animal, while Joachimoglu<sup>53</sup> has demonstrated that in anaphylactic shock the precipitins immediately disappear. The disappearance of precipitins, which no doubt is due to their combination with the antigen, would probably bring about conditions favorable to protease activity, as Bulger<sup>54</sup> and others have shown that proteolysis in serums is active following the formation of precipitates. It is well to bear in mind the possibility that the changes in viscosity following the intravenous injection of nonspecific substances may cause a combination of the antigen and antibody which is

50. Wright, A.: *Brit. Med. Jour.*, 1915, **1**, 625.

51. Rettgers, L., Berman, N., and Sturges, W.: *Jour. Bacteriol.*, 1916, **1**, 15.

52. Weil, R.: *Jour. Immunol.*, 1916, **1**, 47.

53. Joachimoglu, G.: *Ztschr. f. Immunitätsforsch.*, 1911, *Orig.*, **8**, 453.

54. Bulger, H.: *Jour. Infect. Dis.*, 1916, **19**, 882.

already present, and thus duplicate the conditions produced when additional antigen is introduced.

Similar changes may occur in the serum of patients with acute general infections when they are treated with nonspecific substances. Under these conditions it is not difficult to understand how areas of lowered antiferment content would be obtained in which proteolysis would occur. In such instances we may have a temporary increase in intoxication owing to the hydrolysis of the native bacterial proteins to toxic substances, and then a detoxication due to their further hydrolysis. This may be the explanation of the chill which is followed by a drop in temperature, that occurs after intravenous injections of various substances. These changes in viscosity may also help to explain the fall in antiferment strength which follows the injections.

As stated before, however, these serum changes are more or less temporary in character, and will therefore not explain the permanent recovery of patients treated in the early stages of such diseases as typhoid fever.

#### CONCLUSIONS

According to our present views the symptoms of an infection are the result of the struggle between the infecting bacteria and their toxins, and the protective agencies of the host. Theoretically, then, the results observed might be due either to the destruction of the infectious agent with its products, or to the fact that the cells of the host become resistant to the action of these agents. In either case, from our point of view, the disease ceases to exist. Theobald Smith,<sup>55</sup> in 1910, said: "The effectiveness of vaccines applied in the course of acute febrile diseases, such as typhoid and pneumonia, must be accounted for by principles of which experimental medicine has as yet no definite knowledge," and this view apparently holds true at present.

That the intravenous injection of nonspecific substances exerts a marked influence on those infections in which it has been tried is very evident. It is not believed, of course, that these newer methods will cure all cases of infections. They do, however, open up new possibilities and suggest new methods for attacking infections of unknown etiology, as also those caused by organisms for which we have no specific antisera. That all cases are not benefited does not necessarily reflect on the value of the treatment. There are very few therapeutic measures which do not have the same objection.

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55. Smith, Theobald: *Boston Med. and Surg. Jour.*, 1910, **163**, 275.



# THE EFFECT OF PITUITARY INJECTIONS ON THE BLOOD PRESSURE OF FEBRILE PATIENTS \*

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Although the intravenous injection of pituitary extracts into animals is usually followed by a considerable rise of blood pressure, no comparable effects have been observed after the injection of therapeutic doses of this drug into man. Thus, van den Velden<sup>1</sup> says that he observed no constant and striking rise after injecting the usual doses, and Behrenroth,<sup>2</sup> while noting a general skin pallor after intravenous injections, was unable to establish any definite and constant change in the blood pressure.

Since plethysmographic records of the arm made in this clinic showed that pituitary injections influenced the volume and form of the febrile pulse, it seemed worth while to restudy the effect of such injections in this class of patients in order to determine whether the changes observed in the pulse volume and form were accompanied by alterations in the systolic or diastolic blood pressures.

Twenty-seven observations were made on fifteen patients. Of these, six had pulmonary tuberculosis, three infectious sore throat, one Graves' disease, three lobar pneumonia, one surgical shock, and one was convalescing from typhoid fever. From 1 to 1½ c.c. of extract of the pituitary gland (pituitrin P. D. & Co.) was injected deep into the muscle of the arm. The blood pressures were taken several times before the injection, and thereafter at intervals of about fifteen minutes for a period of one hour or more. The pulse rate, temperature and rate of respiration were also noted. Aside from an occasional slowing of the pulse rate, which never exceeded ten beats per minute, no definite change in these occurred.

Before the injection the pulse was usually of a bounding character (pointed or collapsing pulse). Following the injection it could often be felt to become smaller and more sustained. This change was usually noted in the observation made fifteen minutes after the injection, and

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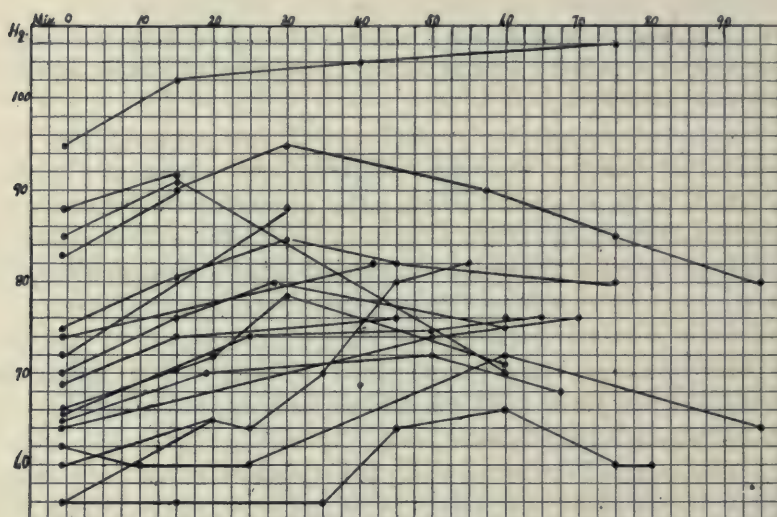
\* From the Department of Internal Medicine, University of Michigan.

1. Van den Velden, R.: Die Nierenwirkung von Hypophysenextrakten beim Menschen, Berl. klin. Wchnschr., 1913, **50**, 2083.

2. Behrenroth, E.: Ueber die Einwirkung des Hirnanhangsextraktes auf den Blutdruck des Menschen nebst Bemerkungen über einige Injektions-versuche am wachsenden Tier, Deutsch. Arch. f. klin. Med., 1913-1914, **113**, 393.

it continued for an hour or more. In certain instances the change in pulse form and size was recorded by a Jaquet sphygmograph, as well as by a small volume recorder attached to a finger plethysmograph, thus demonstrating that the changes in pulse volume and form can be recorded by other methods than the arm plethysmograph.

The systolic blood pressure after pituitary injections was not altered in any constant or striking manner. At times it remained constant, at other times it rose slightly, while at still other times it fell somewhat. The diastolic blood pressure, on the other hand, which was taken by the auscultatory method, and was read at the end of the fourth phase, rose in the great majority of instances (see accompanying chart).



The effect of an injection of pituitary extract on the diastolic blood pressure of febrile patients. Note that as a rule there was a rise at the end of fifteen minutes, and that the maximum effect was observed after from thirty to forty-five minutes.

This rise was usually apparent in the observation taken fifteen minutes after the injection and reached its maximum in thirty to forty-five minutes. It therefore corresponded in time with alterations observed in the volume pulse and with the observed effects on the uterine contractions. The rise in the diastolic pressure amounted in some instances to 15 mm. Hg, or more, and this, together with its time and relative constancy, made it certain that it was due to the action of the drug. The impression was gained, furthermore, that the rise in diastolic pressure was most striking and constant when the pulse was of a definitely pointed form.

No effect on the pulse or blood pressure was observed when the pituitary extract in doses of 2 c.c., or when pituitary substance in doses of 15 gr. of the desiccated gland, was administered by mouth.

In several patients repeated injections of pituitary extract were given at intervals of two hours or more. So far as could be determined, the effects produced by subsequent injections differed in no way from the effects produced by the primary injections.



# DIMINISHED BLOOD PLATELETS AND MARROW INSUFFICIENCY

A CLASSIFICATION AND DIFFERENTIAL DIAGNOSIS OF PURPURA HEMORRHAGICA, APLASTIC ANEMIA, AND ALLIED CONDITIONS \*

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The two conditions, idiopathic purpura hemorrhagica and aplastic anemia, present the common symptoms, anemia and purpura. In text-book descriptions of purpura hemorrhagica and aplastic anemia there is usually no mention of a differential diagnosis between these conditions, though Naegeli<sup>1</sup> does briefly consider the differences, and Frank,<sup>2</sup> in a comprehensive paper, discusses these types of cases. Naegeli<sup>1</sup> and Pratt<sup>3</sup> confirm my own belief derived from clinical discussions, from the study of cases, reports and text-book descriptions, that these conditions are not infrequently confused with each other. Not only are these two conditions confused with each other, but also with the nonleukemic phase of leukemia, bone marrow tumors, osteosclerosis, and, at times, with pernicious anemia and types of splenic anemia, etc.

Purpura hemorrhagica is sometimes confused with the other types of purpura, and the chronic type is often undoubtedly confused with hemophilia. This confusion seems to arise from the lack of a careful study of the blood, so that from some reports (for example, those of Dean,<sup>4</sup> Phillips<sup>5</sup> and Elliott<sup>6</sup>) one cannot be sure of the real nature of the case reported.

The facts and thoughts expressed in this paper have been derived, not only from the literature, but also from a study of some twenty-five cases in the purpura hemorrhagica and aplastic anemia group, all of which have shown few blood platelets. Many of the cases have been studied in conjunction with Dr. Roger I. Lee, to whom I am much indebted. A few of these cases are briefly summarized at the end of the paper.

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1. Naegeli: *Blutkrankheiten und Blutdiagnostik*, Leipzig, 1912.

2. Frank: *Berl. klin. Wchnschr.*, 1915, **52**, 961; also 454 and 490.

3. Pratt: *Osler and McCrae, Modern Medicine*, 1915, **4**, 687.

4. Dean: *Brit. Med. Jour.*, 1907, **2**, 815.

5. Phillips: *Cleveland Med. Jour.*, 1915, **14**, 533.

6. Elliott: *THE ARCHIVES INT. MED.*, 1909, **3**, 193.

## PURPURA HEMORRHAGICA

Purpura hemorrhagica may be considered as a condition in which we have bleeding from one or more mucous membranes, often with purpuric skin lesions, associated with a diminution of blood platelets, a delayed bleeding time, and a nonretractile soft blood clot. The coagulation time is normal or slightly delayed, rarely much delayed. Purpura hemorrhagica is perhaps more usually secondary (Pratt,<sup>3</sup> Matthews and Carpenter<sup>7</sup>) to some recognized disease, as diphtheria or tuberculosis, and likewise is considered secondary when it accompanies aplastic anemia, leukemia, bone marrow tumors, pernicious or splenic anemia, etc. In such conditions it is a symptom. When it is not secondary to a recognized disease, it then constitutes what is often regarded as a disease entity called purpura hemorrhagica, the synonyms of which apparently are essential thrombopenie, pseudohemophilia, and sometimes Werlhof's disease.

Idiopathic purpura hemorrhagica is a disease that may be acute, subacute or chronic, of a continuous or intermittent nature—in some instances lasting over twenty years. A congenital and hereditary type exists.

Purpura hemorrhagica has been especially studied by the French, notably by Hayem<sup>8</sup> and Bensaude and Rivet.<sup>9</sup> In this country Coe<sup>10</sup> first reported cases of the chronic type. Duke<sup>11</sup> has made careful studies on purpura hemorrhagica clinically and experimentally.

## APLASTIC ANEMIA

The term acute aplastic anemia, as a disease entity, should be reserved for a disease whose etiology is unknown and which runs an acute, progressively downward, fatal course of about three to six weeks' duration, occurring especially in individuals between 15 and 30 years of age. Fever frequently occurs. There is no evidence of increased blood destruction, as in pernicious anemia. Whatever the cause of the disease may be, we are bound to suppose that its chief effect is to inhibit the formation of all new elements from the bone marrow, for at necropsy the marrow of typical cases shows practically complete fatty degeneration.

Aplastic anemia has become best recognized as a condition simulating, but to be sharply differentiated from, Addisonian anemia (pernicious anemia) and the so-called degenerated secondary anemias. The

7. Matthews and Carpenter: *Am. Jour. Med. Sc.*, 1911, **143**, 36.

8. Hayem: *Presse méd.*, 1895, p. 233.

9. Bensaude and Rivet: *Arch. gén. de méd.*, 1905, **1**, 193.

10. Coe: *Jour. Am. Med. Assn.*, 1906, **47**, 1090.

11. Duke: *THE ARCHIVES INT. MED.*, 1912, **10**, 445; *ibid.*, *Jour. Am. Med. Assn.*, 1910, **55**, 1185.

papers on this condition by Musser, Jr.,<sup>12</sup> Cabot,<sup>13</sup> Lavenson,<sup>14</sup> Heubner<sup>15</sup> and Larrabee<sup>16</sup> are noteworthy. But as a terminal event in ordinary pernicious anemia the marrow may become exhausted or a terminal aplasia occur. In such an instance we should refer to a secondary aplasia. Aplasia of the marrow resulting from an osteosclerosis or infiltration of the marrow with leukemic or tumor cells, falls into another class and will be referred to later.

During the course of certain recognized infections, as in various types of sepsis, malignant endocarditis, typhoid fever, diphtheria, miliary tuberculosis, and more rarely in pneumonia, aplasia or exhaustion of the marrow may occur, which contributes to the fatal termination of such a case. In such instances we should speak of a secondary aplastic anemia, as we do a secondary purpura hemorrhagica, because we recognize some existing disease in the course of which aplasia or exhaustion of the marrow develops. It is perhaps best to consider that such an aplasia is due to some peculiar nature of the toxin produced by the organism causing the recognized disease in the individual case, and perhaps coupled with some congenital weakness of the marrow as suggested by Webber.<sup>17</sup>

The difference between the secondary and idiopathic cases of aplastic anemia may be summed up by saying that in the former we recognize a source for toxin formation and in the latter we do not. The blood picture in both may be identical and at times the character of the marrow at necropsy. In cases exhibiting sepsis, especially those with oral sepsis, we must distinguish the resulting anemia of a septic infection and the sepsis occurring from the lowered resistance of the body due to the aplasia. The latter is the type occurring in the idiopathic cases of aplastic anemia.

#### BLOOD PICTURE OF APLASTIC ANEMIA

In aplastic anemia the blood picture shows no evidence, or very little evidence, of regeneration of the red cells, platelets and polynuclear leukocytes; that is, of any of the three formed elements of the blood that originate in the bone marrow. The red cells appear quite well filled with hemoglobin. The color index averages 0.8,<sup>13</sup> often slightly higher. There is little or no variation in shape and little variation in size. Polychromatophilia, stippling, blasts, reticulation of red cells and Howell-Jolly bodies are absent or rare. Thus the red cells in an

12. Musser: *THE ARCHIVES INT. MED.*, 1914, **14**, 275.

13. Cabot: Osler and McCrae, *Modern Medicine*, 1908, **4**, 637.

14. Lavenson: *Am. Jour. Med. Sc.*, 1907, **133**, 100.

15. Heubner: *Folia haemat.*, 1915, **19**, 347.

16. Larrabee: *Am. Jour. Med. Sc.*, 1911, **169**, 57.

17. Webber: *Proc. Roy. Soc. Med.*, 1914, **7**, 184.



ordinary stained smear appear not much altered from normal, which is a rather striking fact when the red count and hemoglobin are found very low, the red count often reaching 500,000 before death. In any other condition, with such low red counts as occur in the course of aplastic anemia, we find a greater change in the cells from normal. Should the color index be high, and should we find an occasional and unduly large, and especially an oval shaped, erythrocyte, but the other characteristics of aplastic anemia, we should suspect that though we had a case of aplasia of the marrow, it had developed from a case of pernicious anemia, as Musser<sup>12</sup> has pointed out.

The platelets in the blood in cases of aplastic anemia are markedly diminished, often nearly absent. Their character has not been reported on, though it has been noted<sup>18</sup> that in general when platelets are few they are usually large. In one case, which I have examined with this point in mind, they seemed to be often abnormally large; while in another case they were usually about normal size, occasionally small and some were definitely large, but not markedly so. The white count shows a leukopenia more marked as the disease progresses. This leukopenia consists in an absolute diminution of the polynuclear cells, and usually in disappearance of eosinophils, with thus a relative but not absolute lymphocytosis. The lymphocytes in my five cases like other cases (Musser,<sup>12</sup> Cabot<sup>13</sup>) varied from 45 per cent. to 92 per cent., averaging 73 per cent. They remain of the normal type. I have found no reports on the character of the polynuclears. The few polynuclears I was able to observe in three cases showed in each case normal types. It seemed, however, as if the single and double lobed types were more plentiful than normal, so that there occurred a "left-handed shift" (Arneth), which is in contrast to the frequent "right-handed shift" seen in pernicious anemia as pointed out by Briggs.<sup>19</sup>

#### THE BLOOD IN CLEAR CUT CASES OF PURPURA HEMORRHAGICA

The only constant and striking feature in the blood of cases of purpura hemorrhagica is the marked reduction of blood platelets. I have recently described in detail a typical case.<sup>20</sup>

The blood picture in typical cases of purpura hemorrhagica is consistent with a bone marrow which is unable to form platelets, but to a greater or less degree is able to produce polynuclear leukocytes and red cells.

The anemia in these cases is largely due to the hemorrhage and not apparently to a lack of formation or to an increased destruction

18. Kemp, Calhoun and Harris: Jour. Am. Med. Assn., 1906, **46**, 1022.

19. Briggs: Am. Jour. Med. Sc., 1914, **148**, 413.

20. Minot: Am. Jour. Med. Sc., 1916, **152**, 48.

of red cells. The red cells accordingly are of the type found in an acute or chronic posthemorrhagic anemia — variation in shape is slight, variation in size is evident—some achromia occurs, and the color index tends to be low. Polychromatophilia and stippling occur, at times a few normoblasts, and apparently rarely a Howell-Jolly body. The reticulated red cells, which seem to be an excellent indicator of the red cell activity of the marrow, are increased when definite anemia is present. The white count is somewhat elevated, usually about 12,000, rarely as high as 35,000.<sup>3</sup> An increased percentage of polynuclears occurs with the higher and often with the lower counts. It seems that the brisker the hemorrhage the more response there is of the polynuclear elements of the bone marrow, and likewise of the red cell elements.

In four cases of purpura hemorrhagica I have noted the type of polynuclears. Twice there was a definite "left-handed shift" (Arneth), and twice this picture was about normal, or perhaps a slight "left-handed shift" occurred.

The platelets in normal blood range from about 225,000 to 325,000 per mm.<sup>21</sup> In purpura hemorrhagica they are diminished, and when below about 60,000, hemorrhages tend to occur. The platelets are often markedly diminished (1,000) when the bleeding becomes severe. This is quite opposite to the marked increase, frequently to 1,000,000, seen in ordinary posthemorrhagic anemia where the blood picture is one of active bone marrow regeneration for all the three formed elements. The character of the few platelets to be seen in purpura hemorrhagica has been noted by Hess<sup>22</sup> to be both abnormally small and abnormally large. I have also noticed in such cases unusually small and unusually large platelets. However, they were not as large as the huge ones seen after splenectomy in pernicious anemia. The study of many bloods suggests that from the size and shape of the platelets we may obtain information on altered bone marrow activity.

BLOOD PICTURE IN CASES SUGGESTING AN INTERMEDIATE TYPE OF BONE MARROW BETWEEN APLASTIC ANEMIA AND PURPURA HEMORRHAGICA AND CONSIDERATION OF SUCH CASES —  
CASES CALLED PURPURA HEMORRHAGICA

Some cases of purpura hemorrhagica do not have an elevated white count. A leukopenia is occasionally present. Coe<sup>10</sup> pointed out that in some of his cases, as has occurred in other reported cases, as Levison's,<sup>23</sup> and cases I have studied, the white count was not greater

21. Wright and Kinnicutt: Jour. Am. Med. Assn., 1911, **56**, 1457.

22. Hess: THE ARCHIVES INT. MED., 1916, **17**, 203.

23. Levison: Jour. Am. Med. Assn., 1906, **47**, 936.



than 5,000 to 6,000, and not as much elevated as a similar amount of direct hemorrhage would cause in an individual whose hemopoietic system was functioning normally. Such cases suggest that the bone marrow could not respond as well to the stimulus received by the hemorrhage as it should, and suggest, further, that there may have been some depression of the white cell elements of the marrow. This, of course, suggests a transition towards an aplasia that involves the platelets markedly, the white cell elements of the marrow slightly, but not the red cell elements, for in such cases regenerative forms are frequent, though not abundant. Again, at times one may see in some cases with a normal or slightly increased white count a slightly increased percentage (50 per cent.) of the lymphocytic cells, which would likewise suggest a lack of response of the polynuclear elements of marrow. Other cases, as Case 5, presenting a chronic anemia without any signs of increased blood destruction and without much loss of blood, show red cells of a character that strongly suggest poor erythrocytic activity of the marrow. The white cells in these cases show a leukopenia with slight relative and absolute diminution of the polynuclears. The platelets are of course diminished in numbers, but only enough to cause mild purpura hemorrhagica symptoms. Such cases often spontaneously get somewhat better, only eventually to relapse. These cases suggest a midway stage between purpura hemorrhagica and aplastic anemia.

We should also note that cases approaching these intermediate cases are those of true aplastic anemia, like one I have recently seen (as evidenced by the blood picture, rapid course of the disease and yellow fatty marrow), that show during the earlier course of the disease less evidence of aplasia than others, as evidenced especially by the presence of a few reticulated cells rather than none, and the presence of a slight leukopenia and slight lymphocytosis rather than a marked leukopenia and marked lymphocytosis. In other words, one can find cases in which the blood picture can be interpreted as showing all degrees of involvement of the three elements of the marrow equally or unequally which belong to this group of cases called purpura hemorrhagica. These intermediate cases seem related to the more clear cut disease known as idiopathic purpura hemorrhagica, in that, in common, the nature of the process is unknown, the most marked symptom is purpura or bleeding, and the most striking blood finding is the diminution of blood platelets. As yet, inadequate data do not permit the assumption, but certainly suggest, that all these conditions with diminished platelets and purpura are essentially similar, even if in some cases the lesion is wholly situated in the marrow and in others is referable to increased platelet destruction in the plasma, which will be referred to later. The disease process is always directed intensely towards the



platelets, at times exclusively and specifically so. In some cases, however, this process acts less specifically and involves to a varying degree the other elements of the bone marrow in addition to the platelets.

Cases of this intermediate group are comparable to the partial aplasia produced in many instances by benzol. The action of this drug seems most marked on the polynuclear elements of the marrow, next on the platelets and the red cells. Cases of benzol poisoning had best be called by this name, as here we have a clearly recognized poison that acts on the marrow, the purpura hemorrhagica becoming a symptom of the poisoning and the aplasia of the marrow the pathology of the condition.

Cases also suggesting an intermediate type of disease between aplastic anemia and purpura hemorrhagica are those of a unique character reported by Larrabee<sup>16</sup> and also by Turk.<sup>24</sup> These are cases having the blood picture of aplastic anemia that remitted, finally to relapse and result in the death of the patient.

It is to be noted that in those conditions which we call typical aplastic anemia the red cells and white cell elements are involved before the platelets, so that there is a definite degree of anemia established before hemorrhage occurs. In the types of purpura hemorrhagica the platelets are involved at the onset, so that the hemorrhage appears early, before the anemia is especially evident. In some cases reported<sup>25</sup> of aplastic anemia the hemorrhage has been the first symptom. But it is not the hemorrhage that has caused the anemia here. Rather, these are cases in which the platelet elements are especially involved early and generalized aplasia immediately follows. Or we can perhaps better explain such an instance by the fact that an acute generalized aplasia will be evidenced first in the platelets, because the life of the platelets is but three to five days, and that of the red cells definitely longer, ten to fourteen days. That is, the platelets circulating in a body not forming new platelets or red cells, will disappear before the circulating red cells.

#### STIMULATION OF THE MARROW IN THESE CASES

One must scrutinize the cases of purpura hemorrhagica and aplastic anemia as one does other cases of "blood disease," not only from the standpoint of marrow activity, but with reference to the degree of stimulation the marrow can receive, for example, from transfusion. One should follow not any of the three formed elements originating in the marrow, but all of them in a similar fashion to that used by Lee,

24. Turk: Quoted by Frank (see footnote 2).

25. Steinhaus and Stordeur: Arch. de méd. exper. et d'anat. path., Paris, 1908, **20**, 205. Hirschfeld: Berl. klin. Wchnschr., 1906, **43**, 544.

Minot and Vincent<sup>20</sup> in their splenectomized cases of pernicious anemia. The study of the absolute and relative numbers of the polynuclear leukocytes, the reticulated red cells and platelets is of special importance in determining the degree of activity of these three formed elements originating in the marrow.

In aplastic anemia there is no evidence of regeneration or activity of the marrow as reflected in the blood picture, and following transfusion there will be no evidence of a stimulation of the marrow by this procedure.

The degree of involvement of the marrow in purpura hemorrhagica cases and the intermediate group of cases can be further judged by the resulting stimulation of the marrow by such a procedure as transfusion.

In some cases of purpura hemorrhagica one sees all of the bone marrow elements favorably affected after transfusion; in other cases, only the red cells and polynuclears in varying degrees. It is to be noted that a *permanent* rise of platelets is best referred to a stimulation of the marrow and not to a filling up of the patient with normal blood relatively rich in platelets. The slight *immediate* rise in the platelets in such cases, following a transfusion with the accompanying temporary cessation of the hemorrhage, is referable to a simple replacing of the lacking element, while a *permanent or a marked rise even if but temporary*, is a sign of an increased activity of the diseased marrow, provided we suppose there is not previously an abnormal rate of destruction of platelets that becomes lessened.

In some cases transfusion will add to the red and white cell elements of the marrow a stimulation beyond that which it has already received by the hemorrhage, yet the platelet elements will be but slightly affected. In other cases, especially those referred to as intermediate types of purpura hemorrhagica, the effect of transfusion on either or both the red and white cells will be slight, suggesting further that the activity of these cells in the marrow is depressed. We may conceive of a partial general aplasia in such instances.

Such cases as Case 5 may be capable of some mild stimulation, but even after transfusion, never exhibit as marked stimulation of the red and white cells as is seen following hemorrhage in some of the more sharply defined types of purpura hemorrhagica.

It is also to be noted that in some instances following transfusion one may see a temporary depression of the marrow, later followed by a stimulation or by simply a return to the state before transfusion. I have seen, for example, three instances of mild secondary purpura hemorrhagica developing in cases of pernicious anemia associated with increased diminution of platelets and increased polynuclear leukopenia

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26. Lee, Minot and Vincent: Jour. Am. Med. Assn., 1916, **67**, 719.



and diminished reticulated cells, some twenty-four hours after transfusion. Also, for example, I have seen a case of idiopathic aplastic anemia, which showed before several transfusions a white count of 2,000 to 3,000, with 50 to 60 per cent. polynuclears and 0.2 to 0.7 per cent. reticulated cells, exhibit evidence of an increased depression of the marrow that was left to functionate, as evidenced by the white count becoming 1,000 to 2,000, the polynuclears 30 to 40 per cent., and the reticulated red cells entirely disappearing the day after transfusion. The bleeding in this case, however, was controlled by transfusion, presumably by the transfused platelets.

In cases of benzol poisoning, if the source of poisoning is removed, we might then expect that as the body cannot hold benzol the marrow would begin to regenerate. This occurs in the mild cases, but in the severer cases it seems the marrow is so severely injured that there is no regeneration, and death ensues. It seems, however, that if cases of the severer type are tided over a period of time by repeated transfusions, recovery can take place. Such an instance is reported by McClure,<sup>27</sup> the same case studied by Minot, Denny and Davis.<sup>28</sup> This suggests that the poison has acted to "spring some trap" that prevents a regeneration unless sufficient time is given for its release or sufficient repeated efforts at stimulation finally allow the marrow to react favorably.

This, in turn, suggests that if one obtains no result from one transfusion in idiopathic purpura hemorrhagica cases, he should, if possible, repeat the procedure. Of course in benzol poisoning, the cause being removed, one may rightly hope for the ultimate restoration of a damaged marrow. In purpura hemorrhagica, since the cause is unknown, success depends on unknown factors.

#### ABNORMAL DESTRUCTION OF PLATELETS AS POSSIBLE CAUSE FOR PURPURA HEMORRHAGICA. CONSIDERATION OF PATHOLOGY

The presence of very few blood platelets in the circulatory blood in many instances can be accounted for by a marked diminution in the megakaryocytes (giant cells) of the bone marrow, cells from which the platelets are formed, as conclusively shown by Wright.<sup>29</sup> Diminished numbers of platelets in the blood stream, however, are not always associated with diminution of megakaryocytes. In such cases we must conceive of some other process than destruction of the megakaryocytes to account for the diminished circulatory platelets. One plausible explanation is that the platelets, as fast as they are formed, are rapidly

27. McClure: Jour. Am. Med. Assn., 1916, **67**, 793.

28. Minot, Denny and Davis: THE ARCHIVES INT. MED., 1916, **17**, 101.

29. Wright: Jour. Morphol., 1910, **21**, 263.



destroyed in the circulating blood.<sup>30</sup> Should this occur, or should there be a destruction of the giant cells, or their ability to form platelets be altered, the cases of idiopathic purpura hemorrhagica would present, as far as we now know, quite the same blood picture.

The few reports that I have been able to locate on the pathology of the marrow in cases exhibiting before death the blood picture of pure idiopathic purpura hemorrhagica have generally simply stated the marrow was reddish. I have not found in the literature any report of a careful histologic study of the marrow in these cases. Dr. Wright has kindly located notes of an unpublished case of idiopathic purpura hemorrhagica which he studied some years ago and has recently gone over with me the fixed preparations from the necropsy. Clinically, the case closely resembled Case 1. The patient, a boy of 14, suddenly began having purpuric skin lesions, followed rapidly by repeated hemorrhages from the mucous membranes and fresh crops of purpuric spots on the skin, until death six weeks after the onset. The platelets were markedly diminished. A progressive anemia of the so-called secondary type developed, with elevated white count and essentially normal percentages of polynuclear cells. The necropsy showed a red marrow, with evidence of active regeneration of the red and white cell elements. Interestingly enough, the megakaryocytes were plentiful, perhaps slightly increased above normal numbers. From the available preparations one could not tell if there was any definitely altered histologic appearance of these cells. The other findings at necropsy were hemorrhages into many organs, an otitis media and streptococcus septicemia. The latter, from the clinical chart, would seem to be entirely secondary. The picture of the marrow in this case is quite in accord with the findings of Lee and Robertson<sup>31</sup> in experimental purpura hemorrhagica caused by antiplatelet serum. These authors found but slight alteration in the character of the megakaryocytes and no diminution in their number. Owing to the rapidity with which the experimental animals developed symptoms of purpura hemorrhagica, it would seem that the cause of the diminished platelets was due to their destruction in the circulating plasma. The presence of a plentiful number of megakaryocytes in the human case would also suggest that the platelets were formed in normal or even increased numbers, but that their scarcity in the peripheral blood was due to an abnormally rapid and constant destruction. Unfortunately, we have no means of determining the rate or degree of platelet destruction, as we have in the urobilin tests for red cell destruction. The congenital and chronic acquired types of purpura hemorrhagica might well be comparable to the con-

30. Foa: Arch. sc. méd., Paris, 1916, **39**, 317.

31. Lee and Robertson: Jour. Med. Research, 1916, **33**, 323.

genital and acquired types of hemolytic jaundice, in the former the platelets being involved in a similar fashion as the erythrocytes are involved in the latter.

We do not have to assume that to produce idiopathic purpura hemorrhagica there must be either a destruction of platelets in the plasma or a specific toxic action to destroy megakaryocytes or bone marrow in general. We can suppose that, though these giant cells are plentiful in numbers, they become affected so that they are unable to allow platelets to be cut off from them in normal fashion. It seems that no matter which of these three pathologic processes may occur, however, and perhaps all three occur singly or together in different cases, the blood picture would be the same as far as any methods now known can tell us.

It may well be that the action of an abnormally rapid destruction of platelets in the blood stream in certain instances acts to cause, to a greater or less degree, a depression of the whole marrow.

#### FURTHER CONSIDERATION OF PATHOLOGY IN THIS GROUP OF CASES

Diminution of the megakaryocytes is undoubtedly the cause of the reduction of platelets in the circulatory blood in aplastic anemia, as diminution of the red and white cell elements of marrow is the cause for the diminished red and polynuclear count. In the typical cases one finds a yellow, fatty or gelatinous gray marrow with either very few normal cell elements present or none at all. There are numerous very interesting cases reported in the literature (Larrabee,<sup>10</sup> Frank<sup>2</sup>), giving apparently the blood picture of aplastic anemia, but without clear cut pathologic marrow. Such cases are a study in themselves and will be briefly referred to. Some of these cases have had but few normal cells in the marrow and have shown lymphoid-like cells. Others have shown mixtures of lymphoid areas in a fatty degenerated marrow. Still others have shown foci of normal, perhaps hyperplastic marrow in some parts, with degeneration in other parts. Such cases present a picture of an incomplete aplasia. In fact, we must remember that many cases eventually terminating with complete aplasia, during the course of the anemia do show often a few young regenerative cells, which are probably formed from the small active foci of marrow attempting to compensate the marked destructive process.

In the symptomatic cases of purpura hemorrhagica associated with leukemia or tumor infiltrations of the marrow, the megakaryocytes have been displaced by the abnormal cells so that they are very few in number, as we might expect from the few platelets in the peripheral blood.



It seems most reasonable to assume that the aplasia of aplastic anemia is caused by some unknown toxin or altered physiologic activity, acting on the whole bone marrow, the red cells being first attacked, the platelets being relatively *later* involved as evidenced by the hemorrhages occurring after the anemia has been present for some time. But in purpura hemorrhagica the platelets seem to be first affected by a specific toxin, so that the hemorrhages begin to occur before the anemia, which is, in many instances, chiefly referable to loss of blood. In other instances, the unknown toxin may eventually act directly or indirectly to cause partial or complete aplasia or exhaustion of the marrow. In those cases of purpura hemorrhagica which have been considered intermediate between purpura hemorrhagica and aplastic anemia, we may conceive of the "toxin" of such a nature that besides involving the activity of the megakaryocytes, it has also, to a certain extent, impaired the activity of either or both the red and white cell elements of the marrow.

One need not conceive of a toxin at work to destroy the marrow in such symptomatic cases of purpura hemorrhagica as those due to leukemia, marrow tumors or pernicious anemia, for here there is, as shown by necropsy studies, a displacement of normal cells by abnormal ones.

In many of the cases of chronic disease where depression or inactivity of the marrow exists, probably no toxin is at work. In such instances we could account for the inability of the marrow to form blood elements by supposing a congenital peculiarity of the hemopoietic system. Such cases as Predtechensky<sup>32</sup> refers to would be examples, and perhaps my Cases 6 and 7. In other instances we may conceive that the marrow of the individual has a low ability to withstand an attack by a toxin that ordinarily induces no such effect. We might perhaps conceive of the marrow depression in some instances resulting from disease of some other organ of the body, similarly as we see in disease of some of the glands of internal secretion, hyperactivity of one depressing the activity of the other. There are certain cases grouped under the title of splenic anemia without evidence of increased blood destruction in which a leukopenia with relative lymphocytosis and diminished platelets occur, and in which a purpura hemorrhagica develops. Removal of the spleen in such instances seems to inaugurate in some manner a stimulation of the bone marrow. One might, as Rendu and Widal<sup>33</sup> and others have suggested, consider that some over-activity of the spleen caused a depression of the marrow.

32. Predtechensky, V. E.: *Russky Vrach*, 1916, **15**, 313; abstr., *Jour. Am. Med. Assn.*, 1916, **67**, 244.

33. Rendu and Widal: *Bull. méd. Soc. d'hôp.*, 1899, **3**, 528.



Is it not possible that at times, with or without hypertrophy of the spleen, its physiologic activity is altered so as to cause bone marrow depression? It is, however, to be noticed that in the conditions called idiopathic purpura hemorrhagica and aplastic anemia the spleen is very rarely palpable and never much enlarged. There is a group of cases described by Kleinschmidt,<sup>34</sup> and also by Babonniex and Tixier,<sup>35</sup> in children, in which there is marked evidence of blood destruction, enlargement of the spleen and aplasia of the marrow at necropsy. Such odd types of blood disease serve to show how we can find almost any combination of factors involving blood destruction and formation, and how we can find examples intermediate between the different more clear cut types of blood disease.

Let it be noted here that the various hypotheses and assumptions given above, as well as those that follow, are simply speculations and theories, and not proved facts.

#### FRAGILITY OF RED CELLS IN APLASTIC ANEMIA AND PURPURA HEMORRHAGICA

The fragility of the red cells to various strengths of sodium chlorid solution in a case of aplastic anemia has been studied by Musser.<sup>12</sup> He found that the cells began to break up in almost the same strength salt solution as they normally do, 0.47 per cent., but that complete hemolysis occurred in a greater percentage of salt solution than normal — 0.40 to 0.37 per cent., instead of 0.34 to 0.30 per cent. In one case we obtained exactly the same figures; in another it began in 0.42 per cent., and was complete in 0.36 per cent. solution. Musser considers the alteration in the maximal resistance to be due to the absence of the young red cells. Pepper and Peet,<sup>36</sup> however, were unable to find any abnormal fragility of the reticulated red cells, cells considered to be young red cells.

In experimental purpura caused by antiplatelet serum, Musser and Krumbhaar<sup>37</sup> have recently observed a decreased resistance of the red cells.

Frank<sup>38</sup> studied the fragility of the reds in one case of purpura hemorrhagica and found hemolysis to begin in 0.46 per cent., and complete hemolysis to occur in 0.28 per cent. solution. I found in a case with considerable anemia that hemolysis began in 0.45 per cent., and complete hemolysis occurred in 0.24 per cent. solution. The same figures were also obtained in a second similar case, while in two others

34. Kleinschmidt: *Jahrb. f. Kinderh.*, 1915, **81**, 1.

35. Babonniex and Tixier: *Arch. d. mal. du coeur*, 1914, **7**, 281.

36. Pepper and Peet: *THE ARCHIVES INT. MED.*, 1913, **12**, 81.

37. Musser and Krumbhaar: *Jour. Am. Med. Assn.*, 1916, **67**, 1894.

38. Frank: *Berl. klin. Wchnschr.*, 1915, **52**, 454 and 490.

with but little anemia, hemolysis began in 0.44 per cent., and was complete in 0.30 per cent. solution. Lenoble<sup>39</sup> refers to a case with decreased fragility, that is, increased resistance. In idiopathic purpura hemorrhagica, when anemia exists, it seems that the fragility of the red cells to varying strengths of salt solution is of the type seen with acute secondary anemia; that is, with an increased maximal resistance, while in idiopathic aplastic anemia the maximal resistance seems decreased.

#### CLASSIFICATION OF TYPES OF PURPURA HEMORRHAGICA AND APLASTIC ANEMIA

It is possible to conceive that aplastic anemia and purpura hemorrhagica are not so very distantly related, as also suggested by Frank.<sup>2</sup> We see *pure* types of purpura hemorrhagica and of aplastic anemia; also all intermediate grades. When we recognize a source for a toxin in any type, we call it secondary, while if the toxin or cause is not recognized, we call it primary, the blood picture being the same in either instance.

It seems that we can divide, and perhaps in the future shall further subdivide, into several groups these conditions involving an unknown agent that certainly acts on the marrow in some instances, and may perhaps at times act in some manner specifically on the megakaryocytes or only to destroy formed platelets in the circulating blood. These groups would appear gradually to merge from one into another. It would seem desirable at least to consider cases of purpura hemorrhagica and aplastic anemia in the same general group and not to discuss aplastic anemia under "anemias" and purpura hemorrhagica, only with the arthritic and other purpuras.

It seems to me that we can distinguish the following groups of cases, all of which have the platelets involved in some manner, and all of which occur especially in children and young adults.

- |  |   |
|--|---|
| 1. Pure aplastic anemia  | Aplasia of all elements of the marrow; red cells especially involved.   |
| Duration, weeks; always fatal.   |   |
| 2. Purpura hemorrhagica  | Involvement of platelets only due either to interference with their formation, to abnormally rapid destruction or a combination of factors; hyperplastic or normal appearing marrow occurs in some instances. |
| <i>A. Continuous:</i>  |   |
| Acute or chronic; duration few days to months or even years.                                     |   |
| <i>B. Intermittent or relapsing:</i>   |   |
| Duration of attack days or weeks. Number of attacks two or more, over period of months or years. |   |

Either *A* or *B* type may terminate with aplasia or exhaustion of the marrow involving all its elements, or at any time appear as intermediate types. In

39. Lenoble: Arch. d. mal du coeur, 1913, 6, 313.

Type 2 *A* it is to be noted that platelets are always diminished below normal. With attacks of bleeding they become more diminished, or may throughout the course of the disease remain so low that purpuric symptoms are constantly present. In Type 2 *B* the platelets are diminished below normal only with the attacks.

### 3. Intermediate type of bone marrow involvement usually called purpura hemorrhagica

This may be the end-result of Type 2, or appear as the subacute or chronic form. Duration, weeks, or more; usually over months or years; may be intermittent in the intensity of the involvement of the marrow elements.

Platelet elements of marrow involved with varying degrees of depression or aplasia of either or both the white and red cell elements of the marrow. Perhaps some platelet destruction also occurs.

Purpura hemorrhagica is hardly a satisfactory name for the disease conditions referred to — the term does well for the name of the symptoms. Aplastic anemia is a satisfactory term, but it is a term to be used only in one special group of these related cases. It would seem better if we chose more specific terms, as chronic insufficiency of the blood platelets, or, congenital hypoplasia of the marrow. Best of all, I think, is *insufficiency of the marrow with especial involvement of the platelets or other formed elements in varying degrees*. Also we should very probably refer to some cases as associated with abnormally rapid platelet destruction.

## DIFFERENTIAL DIAGNOSIS OF PURPURA HEMORRHAGICA AND APLASTIC ANEMIA

We may recall here that certain poisons, as benzol, act to produce varying degrees of aplasia of the marrow, and we should not forget the possibility of such poisoning when cases of an aplastic or hemorrhagic type present themselves. Such instances as those in which we recognize the etiology should be called by the name of the poison producing the diseased condition. Purpura hemorrhagica and aplastic anemia may appear as idiopathic diseases or as conditions secondary to some recognized disease. Let us briefly consider the diseases with which purpura hemorrhagica and aplastic anemia are more usually confused.

1. *Other Types of Purpura*.—Purpura hemorrhagica is not to be confounded with certain other types of purpura, drug purpuras, purpura simplex, some cases of so-called Henoch's purpura, and purpura rheumatica (Schoenlein's purpura). These conditions differ in various ways from purpura hemorrhagica, but especially in that *their blood platelets occur in essentially normal numbers*, and sometimes above normal. It may also be noted that joint symptoms in purpura hemorrhagica are very rare. It seems to me that improper use of the term



purpura hemorrhagica is not infrequent; for example, Bauch<sup>40</sup> (1916) entitles an article, "Three Cases of Purpura Hemorrhagica in Chronic Tuberculosis." None of these cases had diminished blood platelets; they resemble more cases of arthritic purpura. These cases are not like the cases of purpura hemorrhagica in tuberculosis described by Bensaude and Rivet<sup>41</sup> in 1906.

2. *Hemophilia*.—In hemophilia the blood platelets are in normal numbers or slightly increased, though they are physiologically and qualitatively defective, as pointed out by Fonio,<sup>42</sup> and Minot and Lee.<sup>43</sup> The differential diagnosis between hemophilia and purpura hemorrhagica has been recently discussed by Hess<sup>22</sup> and Minot and Lee.<sup>43</sup> In hemophilia the coagulation time and prothrombin time of the blood is nearly always much delayed; in purpura hemorrhagica these times are rarely more than slightly delayed, and are often normal. The blood clot in hemophilia retracts in normal fashion, while in purpura hemorrhagica it does not. The bleeding time in hemophilia is essentially normal, in contrast to the delay, often marked delay, seen in purpura hemorrhagica. It is to be noted, however, that the chronic types—especially the congenital types of purpura hemorrhagica—are usually incorrectly diagnosed as hemophilia. When one hears of a diagnosis of hemophilia in females, probably the correct diagnosis is purpura hemorrhagica, as true hemophilia occurs only in males. Females of hemophilic families may, however, present this chronic type of purpura hemorrhagica. It seems that the chronic type of purpura hemorrhagica is probably more common than is usually recognized; and also that the conditions involving varying degrees of bone marrow insufficiency are probably more common than one would be led to believe from textbooks and the literature. It is quite frequent to have one's attention first attracted to these conditions by removal of teeth or minor operations on the nose and throat. With unexplained hemorrhage one should always ascertain the number of platelets and the ability of the clot to retract.

3. *Nonleukemic Phase of Leukemia*.—Purpura hemorrhagica and aplastic anemia are undoubtedly frequently confused with the nonleukemic phase of leukemia, when such a case presents anemia and purpura hemorrhagica as symptoms. The resemblance and the differential diagnosis between these two conditions do not seem to be commented on in most textbooks, though Hastings,<sup>44</sup> Lavenson,<sup>14</sup> and

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40. Bauch: THE ARCHIVES INT. MED., 1916, **17**, 444.

41. Bensaude and Rivet: Presse méd., 1906, **14**, 469.

42. Fonio: Cor.-Bl. f. schweiz. Aertze, 1915, **45**, 1505.

43. Minot and Lee: THE ARCHIVES INT. MED., 1916, **18**, 474.

44. Hastings: Am. Jour. Med. Sc., 1905, **129**, 787.

Naegeli,<sup>1</sup> among others, have pointed out that cases are reported as Werlhof's disease (purpura hemorrhagica) which are probably nothing more than cases of acute lymphoid leukemia. In such leukemic cases the diminution of the platelets and red cells and white cell elements of the marrow is not due to a fatty condition of the marrow, but rather to a crowding out of the normal cells of the marrow by the tumor-like cells (myelophthisis). Usually such cases show at necropsy a red, or partly red, marrow with infiltration by the tumor-like cells. That part of the normal marrow that is left seems still capable of regenerating the red cells, white cells and platelets after normal fashion, though the amount of these elements regenerated is less than normal. Hence, anemia results, purpura hemorrhagica results, and a diminished absolute polynuclear count results. Thus, in the peripheral blood we see a picture of active compensating function of the remaining erythroblastic marrow, that is, polychromatophilic cells, reticulated cells, blasts, and cells of abnormal shape and size. Part of the picture may be due to an irritation of the marrow by the abnormal or foreign cells.

This red cell picture is in contrast to that seen in aplastic anemia and is more like that seen in purpura hemorrhagica. However, some cases exhibiting the picture seen in aplastic anemia, show at necropsy a marrow with few or no normal cells, but with a hyperplasia of lymphoid cells or a lymphoid marrow. Some of these cases remain puzzles; others perhaps are the end-results of a leukemia. Such cases would be perhaps impossible to tell from an aplastic anemia with the usual fatty or degenerate marrow unless one found numerous abnormal types of white cells in the blood smear.

In considering the differential diagnosis of purpura hemorrhagica and the nonleukemic phase of leukemia we should pay especial attention to the presence or absence of the swelling of the lymph nodes. If enlarged, they certainly suggest a leukemic nature of the disease. But unfortunately they are often not appreciably enlarged in the nonleukemic phase of leukemia, so that mistakes in diagnosis are easy.

Naegeli<sup>1</sup> has pointed out that sometimes in the acute cases the lymphocytosis is not very marked, so that the diagnosis must often depend on the demonstration of qualitative variations of the lymphocytes, especially the presence of large lymphocytes with pale nuclei, and on the demonstration of forms with lobulated nuclei described by Rieder and other abnormal types of cells of the lymphocyte series. The finding of a considerable number of abnormal lymphocytes is certainly of great importance in the diagnosis. In most cases, however, it seems that the lymphocytosis is apt to reach a much higher percentage (95 per cent.) than it usually does in aplastic anemia, in which the lymphocytes are normal small lymphocytes. If there is a very



high percentage of lymphocytes in aplastic anemia the red cells are markedly aplastic in character, while with a similar percentage of lymphocytes in a leukemic condition, with a low white count, the reds almost always show some evidence of regeneration. Those cases showing blood of aplastic character with lymphoid marrow are probably very rare. Some of these may be cases of "lymphoid" leukemia of myelogenous origin in the nonleukemic phase.

4. *Bone Marrow Tumors, Osteosclerosis*.—Bone marrow tumor, primary or metastatic, may be the proper diagnosis in a case when anemia and purpura hemorrhagica are the presenting symptoms. The clinical history, physical examination, Roentgen-ray findings and the occurrence of Bence-Jones protein in the urine in myeloma are of great help in distinguishing such cases from idiopathic purpura hemorrhagica. The blood picture may be quite similar, but the presence of abnormal cells, plasma cells, tumor cells, should they occur in the peripheral blood, may suggest the diagnosis. In such cases the liver and spleen may revert to their embryonic state so that hematopoiesis occurs in these organs. I am inclined to believe, especially from Dr. Wright's necropsy studies, that this does not occur in those idiopathic conditions we call aplastic anemia and purpura hemorrhagica.

5. *Pernicious Anemia*.—The diagnosis of pernicious anemia from aplastic anemia is usually simple. If, however, a case of pernicious anemia is first seen towards the ends of the disease when the bone marrow is exhausted, the blood may present a picture resembling that of idiopathic aplastic anemia. The history of remissions, of sore tongue, of symptoms of spinal cord involvement, of a skin colored to suggest increased red cell destruction, would all strongly suggest pernicious anemia and not idiopathic aplastic anemia. The blood picture in those cases of pernicious anemia that become of an aplastic nature will show leukopenia, lymphocytosis and few platelets, but one should note that the color index tends to remain high, and very abnormal shaped and large cells, though perhaps few, are found, rather than the much more normal looking cells seen in true, idiopathic, aplastic anemia.

6. *Splenic Anemia*.—As has been pointed out before, some cases of splenic anemia presenting few blood platelets with their anemia and no, or slight, evidence of increased red cell destruction, may be perhaps quite closely related to types of purpura hemorrhagica. In fact, if it was not for definite, often marked, enlargement of the spleen, and, later in the disease, enlargement of the liver, some cases would be indistinguishable from types of purpura hemorrhagica. Our Case 5, called an intermediate type of purpura hemorrhagica, shows a slightly enlarged spleen, and such a case may well represent a type intermediate between those cases called purpura hemorrhagica and those called



splenic anemia, in which the enlargement of the spleen is greater and precedes the anemia, which in turn becomes quite evident as a rule before the purpuric symptoms appear in these cases.

#### REPORT OF CASES

The following selected, briefly summarized cases serve to show examples of some of the conditions referred to in the text. Case reports of the five cases of typical aplastic anemia studied are not given, as such cases are discussed in the text and the reported cases are entirely similar. These five cases occurred in girls between the ages of 9 and 19. Three cases came to necropsy and showed the typical fatty and often serous degeneration of the marrow, with either very few or no marrow cells left. Some showed a blood picture of very severe aplasia. Others, though typical aplastic anemia, showed a few young red cells and the lymphocytosis was not high (45 to 60 per cent.), indicating that a little regenerative power of the marrow was left.

In the cases given below the complete findings are not given, but only the essential and important ones. Blood cultures and Wassermann reactions were negative in all.

CASE 1.—(W. M. 207893). *An acute idiopathic purpura hemorrhagica with fatal course. It is entirely similar to the one referred to in the text in which necropsy showed a normal or slightly hyperplastic marrow.*

A girl, aged 15, entered the hospital April 19. The history is negative except for the following: Seven weeks before entrance a tooth was pulled. After this there was oozing of blood from the tooth socket for two weeks. Four weeks before entrance her nose began to bleed and bleeding from the gums recurred. During the previous two weeks there had been excessive vaginal flow, and for about the same length of time purpuric eruption on the body. She had grown steadily paler and weaker. The patient died, April 27. While in the hospital blood oozed from all mucous membranes of the body. Only slight temporary improvement followed transfusion of 400 and 600 c.c. of blood. It is noteworthy, however, that as in the previously reported case,<sup>20</sup> the purpuric skin lesions decreased markedly after transfusion and no new lesions appeared, though there was only temporary influence on the hemorrhage from the mucosae. *Physical examination* revealed nothing additional of interest. The temperature fluctuated 98 to 101 or 100 to 103 F. each day.

Blood: The platelets were always very rare, or rare and of a small or normal type. There was no evidence of stimulation of these elements after transfusion. The coagulation time was normal, the clot nonretractile and soft. The bleeding time was much prolonged (often over one hour), though temporarily shortened after transfusion (three to eight minutes). The white count averaged 8,000, the polynuclears 78 per cent., lymphocytes 21 per cent., eosinophils 1 per cent. Arneth count showed a "left-handed shift."

The red count was 1,300,000 to 1,600,000. Hemoglobin 25 to 40 per cent. The red cells showed achromia, considerable variation in size and some variation in shape. Occasionally a blast was seen. Polychromatophilia and stippling were much in evidence. The reticulated cells, chiefly of a knotted type, varied from 14 to 3 per cent. The urobilin excretion was normal.

CASE 2.—(E. M. 184907). *A case of idiopathic intermittent purpura hemorrhagica. Death in the second attack. No signs of depression in the red or white elements of the marrow.*

Man, aged 33; entered hospital September 12. Four years prior to entrance the patient had an attack, lasting about three weeks, of bleeding from the mucous membranes, with purpuric spots on the skin. Eight years previous to entrance he had abdominal pain, diagnosed as plumbism. Nine days before entrance bleeding began from the nostrils, three days before from the gums, and in the previous two days there had been blood in the urine. For a week there were purpuric spots on the skin.

The patient, growing progressively more anemic, died October 14. Recurring crops of purpuric spots occurred on the body, with fluctuations in the intensity of the bleeding from all mucous membranes. The patient was not transfused. *Physical examination* never revealed any abnormalities except anemia and hemorrhages and a functional systolic murmur. The temperature during the first week was 103 to 105 and in later weeks 98 to 100 F.

The blood examination revealed practically the same findings as in Case 1, except that the coagulation time was eighteen minutes. (Normal being five to ten minutes.)

CASE 3.—(W. M. 197121). *Secondary (?) acute purpura hemorrhagica with recovery. Especially interesting because of the fact that ivy poison may well have been the toxin responsible for the diminution of platelets.*

A woman, aged 25, entered the hospital August 15, with evident severe dermatitis venenata of five days' duration, due to poison ivy. August 13 a few purpuric spots appeared on the body and increased in number until August 16, when they rapidly left and were essentially gone by August 19. August 14 and 15 there was slight oozing from the gums and nostrils and blood was found in the stools and urine on the 15th. The physical examination was negative except for the findings noted above. Temperature 99 to 100 F. The patient remained well during the two years following the purpuric attack just described.

Blood: The red count, hemoglobin and red cells were normal. The white count, 12,000, and polynuclears 72 per cent.; the other white cells were chiefly normal, lymphocytes. The platelets numbered 40,000 August 15, and 176,000 August 18. The coagulation time, August 15, was very slightly delayed, but the clot was nonretractile and exhibited the reclothing phenomena.

CASE 4.—(Private Case). *Idiopathic intermittent purpura hemorrhagica. Depression of the white cell elements of the marrow and probably of the red cell elements in the sixth attack. No evidence of white or red cell depression in previous attacks.*

Man, aged 28; the man had good health until five years prior to being seen, when five days after removal of his tonsils he bled profusely from his mouth and nose for three days. In the course of seven years he had six attacks of severe bleeding from his gums and nose lasting three to twelve days. Once a few red cells were found in his urine and a few purpuric spots appeared on his body. The patient was seen in the fourth and sixth attacks. Following each attack a considerable pallor developed. His color became normal about four weeks after each attack except after the last one. Following the fourth attack the red cells rose from 2,700,000 to 4,800,000 in three weeks, but following the last attack the reds rose only from 2,800,000 to 3,700,000 in eight weeks. The patient himself noted that he did not regain his color as easily as after his previous attacks. Unfortunately, he has been lost track of. In both attacks the platelets were very scarce, increasing as the bleeding ceased. They were normal in numbers when twice observed between attacks. In the fourth attack the white count was 14,000 and the polynuclears 78 per cent., in contrast to counts close to 5,000 and polynuclears 50 per cent., with practically all the other white cells small lymphocytes in the sixth attack. The red cells in the fourth showed 13 per cent. of reticulated cells and the other evidences of a very active regeneration of red cells, in



contrast to the red cells seen in the last attack. In this last attack the red cells gave some evidence of regeneration as shown by 3 per cent. of reticulated cells, a few polychromatophilic and stippled cells, and variation in size of cells, but none of the signs of active regeneration were as marked as in the previous attack, which is likewise evident from the history given above of a much slower disappearance of his pallor.

CASE 5.—(E. M. 208209.) *A case of intermittent intermediate type of purpura hemorrhagica. Blood picture suggests some aplasia or exhaustion of not only the platelet elements of the marrow, but also the white cell elements and red cell elements. The anemia presented is not wholly explained by hemorrhage. The lack of evidence of increased blood destruction, history of the patient, and the blood picture suggest that this is not a case of pernicious anemia. What part the enlarged spleen plays in the case, the patient being a Greek, is problematical.*

A Greek boy, aged 19. Seven years prior to admission the patient had painless hematuria for two days; none since. The gums bled at that time and he thinks he may have had some "red spots" on his body. At that time he was in bed for six months on "account of weakness."

The history of his present illness showed that fourteen, eleven and five weeks prior to entrance he had a profuse nosebleed and lost at least a cupful of blood each time. For the previous five weeks he felt weak and dizzy. There was no history of sore mouth, paresthesia, gastro-intestinal symptoms or malaria.

On entrance, May 5, the physical examination showed pallor, a few small purpuric spots on the legs and a palpable spleen (dulness  $10\frac{1}{2}$  by 16 cm.). Otherwise the examination was entirely negative. The patient remained in the hospital until May 23, during which time he occasionally had slight epistaxis and slight bleeding from his gums, until he was transfused with 850 c.c. of blood, May 17, after which he had no more bleeding before he left, May 23. Temperature 99 to 98 F. I have unfortunately not been able to obtain any trace of this patient since he left the hospital.

Blood: The platelets were always diminished; 35,000 on one occasion, before transfusion, and 120,000 on discharge. The bleeding time on entrance was as much as one hour, usually four to eight minutes. The white count varied from 3,600 to 2,100. The polymorphonuclear neutrophils remained close to 54 per cent., the remaining cells were lymphocytic of normal type, except for an occasional transitional cell. Following transfusion the polymorphonuclears reached 66 to 60 per cent., but without any appreciable rise in the white count. The red cells remained about 1,850,000 and the hemoglobin 45 per cent. The red cells showed moderate variation in size, some variation in shape, some macrocytes, but not of an extreme type and not large oval cells. The larger cells were well filled with hemoglobin; the others showed some achromia. Very rarely a stippled or polychromatophilic cell occurred. Only one normoblast was seen in nine different examinations. The reticulated red cells were never over 2 per cent., except five days after transfusion, when they were 3 per cent. They were rarely of a knotted type and usually the reticulum consisted of but scattered strands.

CASE 6.—(W. M. 198899.) *Congenital continual purpura hemorrhagica. Not hemophilia. The circulatory blood platelets were always diminished. Bleeding from various parts occurs readily. With a downward fluctuation of the platelets they become so few that spontaneous hemorrhage occurs.*

The patient was a boy, now aged 12 years, who had been studied many times. Since he was an infant he had had innumerable attacks of bleeding from the nose, throat, gums, rarely gastro-intestinal tract, and purpuric eruptions on his skin. In the previous two years he had had three mild attacks of bleeding. Whenever he was bruised or cut from even slight injuries he bled very readily and in abnormal amounts. There had never been any joint symptoms. Neither his brothers nor sisters are "bleeders," nor is there any history of "bleeders"



in his mother's or father's family. He had measles at the age of 6, with severe hemorrhagic symptoms.

The physical examination had never revealed anything noteworthy beyond the purpura.

Blood: During five attacks, between 1910 and 1916, the platelets were 13,000 to 50,000. Between attacks the platelets have always been definitely diminished (100,000 to 200,000) though not as low as during attacks. The bleeding time has been delayed with the attacks and often slightly delayed (four to seven minutes) between attacks. The coagulation time has usually been normal, occasionally slightly delayed. The white count has been 25,000 to 8,000, higher after hemorrhages. Two observations between attacks showed 8,000 to 14,000. The polynuclear have varied from 78 to 53 per cent. The other white cells have been chiefly small lymphocytes with no abnormal types of white cells. The red count has varied from 2,500,000 following severe hemorrhage to 4,800,000 some months after the last attack. The red cells have appeared normal, or when anemia was present they have been of the type seen with posthemorrhagic anemia.

CASE 7.—(W. M. 165399.) *Another case of the continual chronic type of purpura hemorrhagica either congenital or acquired as a young child. Has been observed especially by Dr. Roger I. Lee over a period of seven years. This case appears to belong to the intermediate group.*

The patient was a woman aged 26. She presented most of the time, some symptoms of purpura hemorrhagica, with diminished blood platelets. She had had numerous attacks of bleeding.

There had been frequently found a leukopenia, usually 3,000, the white count reaching 6,000 to 7,000 after severe hemorrhages. The polynuclears fluctuated between 43 and 68 per cent., the other cells being chiefly normal small lymphocytes.

There had been a chronic, though often slight fluctuating anemia.

This case suggests a greater involvement of white and red cell elements of the marrow than is seen in Case 6 and may possibly be a type of congenital insufficiency of the marrow involving the megakaryocytes especially, the white cells somewhat, and the red cells in a less degree.

CASE 8.—(W. M. 209500.) *A case of leukemia in the nonleukemic phase as shown by the necropsy findings, the diagnosis being made in life by slight enlargement of the lymph nodes and spleen and by the very high lymphocytosis with many abnormal lymphocytes. The purpura hemorrhagica is secondary. The diminution of platelets is due to the crowding out of the giant cells of the marrow by leukemic cells.*

A boy, aged 7 years, began six weeks prior to entrance to lose his appetite and to tire easily. Weakness with night sweats have gradually increased since and he had grown increasingly pale in the previous four weeks. During the previous week his neck had been swollen and he had had a sore throat, with hemorrhages from his mouth and nose, in the previous three days.

Physical examination showed a pale, poorly nourished boy, with a few purpuric spots, and hemorrhages from his nose and mouth. The tonsils were large and buried; tongue and mouth foul with crusts and exudate; cervical lymph nodes the size of beans and peas; inguinal and axillary nodes easily palpable. The spleen was felt 2 cm. below the ribs. Temperature 103 to 105 F. The patient died three days after entering the hospital.

Blood: Red count 2,000,000 to 1,200,000; hemoglobin, 40 to 50 per cent. The red cells showed moderate achromia, some variation in size, slight variation in shape, an occasional polychromatophilic and stippled cell, and rarely a blast; reticulated cells 4 per cent. The platelets were very few. The white count was 3,400 to 4,200; the polymorphonuclear neutrophils 3 to 4 per cent.; rarely a mast cell. The remaining 96 per cent. of the white cells were lymphocytes, some

54 per cent. of which were abnormal appearing large lymphocytes, some of odd types.

Dr. Wright's important necropsy findings were as follows: A raspberry red marrow which microscopically showed a marked infiltration with cells of the lymphocytic series. There were but few of the normal cells of the marrow, and very rarely was a giant cell found. Other organs of the body also showed infiltrations with cells of the lymphocytic series.

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THE REACTION OF THE CEREBROSPINAL FLUID  
PRELIMINARY REPORT ON HYDROGEN-ION CONCENTRATION AS DETER-  
MINED BY THE COLORIMETRIC METHOD \*

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The chemical and physical constitution of the cerebrospinal fluid does not furnish any basis for the conception that the reaction of this fluid might be different from that of the blood. The inorganic components,<sup>1</sup> chiefly chlorids, phosphates, and carbonates, occur in almost identical amounts in both the blood serum and cerebrospinal fluid. The organic constituents<sup>2</sup> vary considerably, but are generally less in amount than similar substances in the blood, and the presence of amphoteric amino-acids and proteins would tend to preserve a neutral reaction in this fluid, as emphasized by Robertson.<sup>3</sup> The quantities of these substances, however, do not indicate their influence on the reaction of the fluids in which they occur. Their molecular concentration and ionic dissociation are the important factors. Even the physical properties are approximately the same in both the spinal fluid and blood serum. As measured by cryoscopy, the blood serum (Hammarsten<sup>1</sup>) causes a depression of the freezing point of  $\Delta = 0.551$  C. to  $0.561$  C.; the spinal fluid,<sup>4</sup>  $\Delta = 0.566$  C. The normal spinal fluid is equally isotonic with blood serum when tested against red blood corpuscles. In this case, as in that of all body fluids, the hydrogen ion concentration is most intimately related to the dissociable carbonic acid contained in the fluid. The following tabulation, quoted from Mott,<sup>5</sup> gives the generally accepted data on the carbon dioxid content of the spinal fluid.

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	Cerebrospinal Fluid Yield, Per Cent.	Lymph and Serum Yield, by Volume, Per Cent.
By vacuum and heating.....	10	46
By acid and heating in vacuo.....	50	50
Difference, representing carbon dioxid in stable combination .....	40	4

Mott has interpreted these findings to indicate that the carbon dioxid is in more stable combination in the spinal fluid than in the blood. A careful analysis of his procedures, however, throws doubt on the validity of these conclusions. Mott does not give any information about the time elapsing between the withdrawal of the fluid and the determination of its content of carbon dioxid, and his data are insufficient to show whether the 10 per cent. of carbon dioxid per volume obtained by heating in vacuo was more readily or less readily released from the spinal fluid than the 46 per cent. by volume from the blood serum. If, as seems likely from a consideration of the lesser viscosity of the spinal fluid as compared with the blood, this 10 per cent. of carbon dioxid is more subject to escape by diffusion from the spinal fluid, it would have a greater influence on the reaction of this fluid than the more slowly liberated carbon dioxid from the viscous blood serum. It is possible, also, that a major portion of the unstable carbon dioxid of the spinal fluid had escaped before Mott's determinations were begun. The final factor in the relationship between the reaction of the blood and spinal fluid must depend largely on the possibility of an interchange of free hydrogen ions between these fluids. In view of the great diffusibility of the hydrogen ion, it does not seem likely that the choroid plexus and the vascular meninges would obstruct the passage of this ion from the blood into the spinal fluid. Since, therefore, the previous studies on the physical and chemical constitution of the spinal fluid and blood lead to the supposition that the reaction of these fluids should be approximately equal, any reports which point to an opposite conclusion require investigation.

In recent communications, Hurwitz and Tranter,<sup>6</sup> and Weston,<sup>7</sup> say that the hydrogen ion concentration of the spinal fluid averages 8.11, while that of the blood serum, as is well known, ranges normally from 7.6 to 7.8. These authors determined the pH by the colorimetric method of Levy, Marriott and Rowntree, applying the test directly to the spinal fluid and to the dialysate of this fluid. Hurwitz and Tranter, relying on Mott's statement about the stability of the combinations of carbon dioxid in the spinal fluid, apparently made no effort to estimate the hydrogen ion in fluids immediately on their withdrawal from the body, when the interval of time would have the

6. Hurwitz, S. H., and Tranter, C. L.: *THE ARCHIVES INT. MED.*, 1916, **17**, 828.

7. Weston, P. G.: *Jour. Med. Research*, 1917, **35**, 367.

least influence on the escape of the carbon dioxid. Weston states plainly that his determinations were made on "cerebrospinal fluids sent to the laboratory for the usual routine tests." As we will show later, it is absolutely necessary to carry out the determination on the fluid as soon as it is withdrawn from the spinal canal in order to obtain an estimation of the true reaction.

These authors quote the work of Bisgaard,<sup>8</sup> as a substantiation of their findings. An analysis of Bisgaard's statements, however, does not permit this interpretation. Using a borate mixture with phenolphthalein as an indicator, Bisgaard determined the pH on two spinal fluids immediately on withdrawal of the fluid. On page 62 of his report he writes:<sup>9</sup> "Both spinal fluids proved to be more acid than the most acid borate mixture (5.7 borate + HCl), viz., pH 8.10." On page 64 he concludes:<sup>10</sup> "The hydrogen ion concentration of the spinal fluid is greater than the value of pH = 8.10." He thus clearly states that the spinal fluid was more acid than his borate mixtures, and that he was unable by this method to determine the exact reaction. He also noted that after these same fluids had stood for three hours, the reaction became progressively alkaline, becoming a little over 8.1.

#### METHOD OF STUDY

The results on which our report is based were obtained by a study of a series of cases at the city hospitals, Bay View Hospital, and at the Johns Hopkins Hospital. In order to classify these spinal fluids more definitely than was permitted by the clinical diagnoses of the cases, they were subjected to the following tests: cell count, globulin-albumin content, Wassermann reaction, colloidal gold test, and refractive index. As will be seen from the cases listed in our tables, a large number of them were psychiatric patients. These tests, however, gave the most positive evidence obtainable that the spinal fluids of many of these patients were normal. On this basis we have classified as normal only those fluids having a cell count below ten, a negative or slightly positive albumin-globulin test by the Ross-Jones and Pandey methods, a negative Wassermann reaction, and only slight changes in the color of the gold solution in Lange's colloidal gold test (Table 2). By these tests the spinal fluids from epileptics were found to be normal. These, however, were grouped in a separate table (Table 4) for the sake of convenience. Other spinal fluids were placed readily in the pathologic categories, of which these tests are the most reliable criteria.

8. Bisgaard, A.: *Biochem. Ztschr.*, 1914, **58**, 1.

9. Beide Spinalflüssigkeiten erwiesen sich dadurch saurer als die sauerste Boratmischung (5.7 Borat + HCl), d. h. pH 8.10.

10. Die Wasserstoffionkonzentration der Spinalflüssigkeit ist grösser als die dem Werte pH = 8.10 entsprechend.



It had been thought that the colloidal gold reactions were related to the hydrogen ion concentration of the spinal fluid. These studies soon demonstrated that the precipitation of the gold solution was independent of the reaction of the spinal fluid.

Réfractometry was carried out on all the specimens, using an Abbe-Pulfrich direct reading refractometer. With this instrument, the refractive index of normal and pathologic fluids ranged within such narrow limits (1.3348 to 1.3351) that it could not be used as an aid to diagnosis.

#### TECHNIC

In order to determine definitely the reaction of the cerebrospinal fluid under conditions most closely approximating its state in the body, the tests were applied to the fluid as soon as it was withdrawn by lumbar puncture from the spinal canal. The hydrogen-ion concentration was determined by the colorimetric method of Levy, Rowntree and Marriott,<sup>11</sup> directly in the spinal fluid and in its dialysate. The colorimeter used was a standardized instrument prepared by Hynson, Westcott and Dunning under the direction of Dr. Marriott. The pipets and other glassware employed in the tests were thoroughly cleaned and rinsed in triply distilled water. Throughout these tests, tubes of non-sol glass were used, and the water employed was always triply distilled from barium hydrate in the second distillation. The collodion sacs for dialysis were made of Anthony's negative cotton, carefully prepared and tested for leakage.

The procedure was as follows: The spinal fluid was allowed to run from the lumbar puncture needle directly into a large, hard glass test tube and from this it was pipetted into the standard non-sol test tube, 10 mm. inside diameter, for use in the colorimeter. To 1 c.c. of spinal fluid was added 0.1 c.c. of 0.01 per cent. phenolsulphonephthalein in triply distilled water, and this was compared at once with the color standards. The dialysis was performed immediately on 1 c.c. of the cerebrospinal fluid in the collodion sac against 3 c.c. of 0.8 per cent. solution of pure sodium chlorid. After ten minutes, 0.3 c.c. of the indicator were added, and the mixture transferred to a tube of the proper size for the slot in the colorimeter. The alkaline reserve (RpH) was determined according to the method of Marriott,<sup>12</sup> by bubbling through the mixture of spinal fluid and indicator, air purified by passage through sulphuric acid, barium hydrate and calcium chlorid. When a constant color was obtained a reading was taken, from which the alkaline reserve (RpH) was calculated.

Blood was drawn from the arm vein of the patient as soon as the lumbar puncture was completed. Estimations of the hydrogen ion concentration and reserve alkalinity of the blood serum were made, usually within six hours, by the same colorimetric method as that used with the spinal fluids. The inevitable delay in the determinations of the pH of the blood probably accounts for the somewhat low readings which we obtained with these specimens.

In these procedures there are several sources of error. The difference between the shade of color produced when fresh spinal fluid is added to phenolsulphonephthalein and the color standard, makes the comparison somewhat difficult. The fluid assumes an orange tint which has no exact match in the standard tubes. The color intensity, however, allows an approximate comparison to be made. This orange

11. Levy, R. L., Rowntree, L. G., and Marriott, W. McK.: *THE ARCHIVES INT. MED.*, 1915, **16**, 389.

12. Marriott, W. McK.: *THE ARCHIVES INT. MED.*, 1916, **17**, 840.



tint is due to the high content of carbon dioxid in the spinal fluid; it disappears as the carbon dioxid escapes, and reappears on the subsequent addition of carbon dioxid to the fluid. Another source of error is introduced when the spinal fluid is allowed to drop through the air from the lumbar puncture needle and is subsequently pipetted from tube to tube. This exposes a great surface area of the fluid to a gaseous medium in which the carbon dioxid tension is less than that within the body. It is obvious, therefore, that a rapid escape of carbon dioxid will occur at once. The tendency of this loss of carbon dioxid is to render the fluid more alkaline.

To demonstrate the changes in the reaction which occur after the fluid has been withdrawn from the body and allowed to stand in contact with the air, determinations were made at short intervals during six hours. The results of these experiments are summarized in Table 1 and illustrated graphically by Charts 1 and 2.

TABLE 1.—EFFECT OF EXPOSURE TO AIR ON REACTION OF SPINAL FLUID

No.	pH Imme- diate	Hours After Fluid Obtained									
		¼	½	¾	1	1¼	2	3	4	5	6
21	7.9	7.9	8.0	...	8.0	...	8.1	8.2	8.5	8.6	
29	7.7	7.75	...	...	7.85	...	8.0	8.1	8.2	8.3	
31	7.8	7.8	7.9	7.9	7.9	7.95	8.0	...	...	...	8.6
32	7.75	7.75	7.8	7.85	7.85	7.95	8.1	...	...	...	8.6
33	7.75	7.75	7.75	7.8	7.8	7.9	7.95	...	...	...	8.6
34	7.8	7.8	7.8	7.9	7.9	7.95	8.0	...	...	...	8.6
35	7.7	7.7	...	7.8	...	7.8	8.0	...	...	...	8.6
36	7.75	7.75	...	7.9	...	7.95	7.95	...	...	...	8.6
37	7.75	7.75	...	7.85	...	7.9	8.1	...	...	...	8.5
38	7.8	7.8	...	7.95	...	7.95	8.1	...	...	...	8.4
39	7.9	7.9	...	7.9	...	7.95	8.1	...	...	...	8.4
40	7.7	7.75	...	7.8	...	7.9	8.0	...	...	...	8.6

These tables and graphs show that the reaction becomes progressively alkaline after the fluid has been drawn. That this increasing alkalinity is favored by conditions which permit the ready escape of carbon dioxid and the possible absorption by the fluid of alkaline gases of the air, such as ammonia, is shown by a comparison of the charts. When the fluid is kept in a tube closed by a cork stopper, the reaction changes gradually according to the even diffusion of the gases. When, however, the cork is removed for a short time at different intervals, the reaction changes by sudden stages, as shown by Chart 2. This

emphasizes the necessity of determining the reaction of the fluid as soon as it is obtained, and at the same time explains the false values found by Hurwitz, Tranter and Weston.

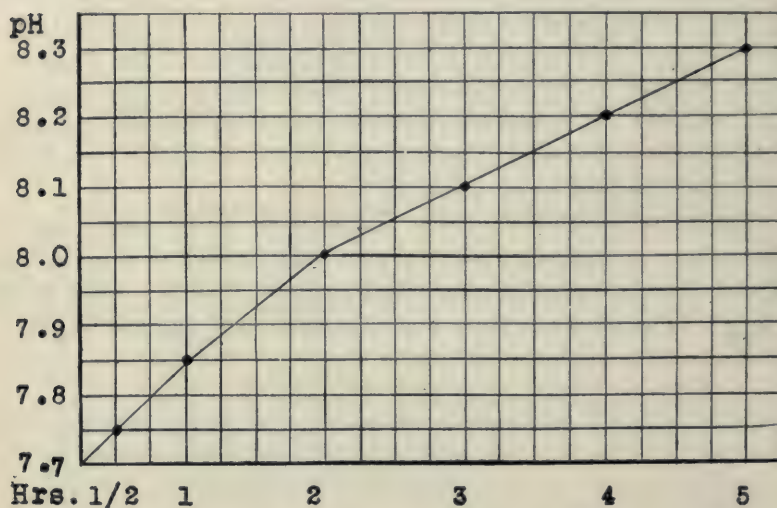


Chart 1.—Spinal Fluid 29-B. Curve shows the increase in alkalinity of spinal fluid kept for five hours in a test tube closed with a cork stopper.

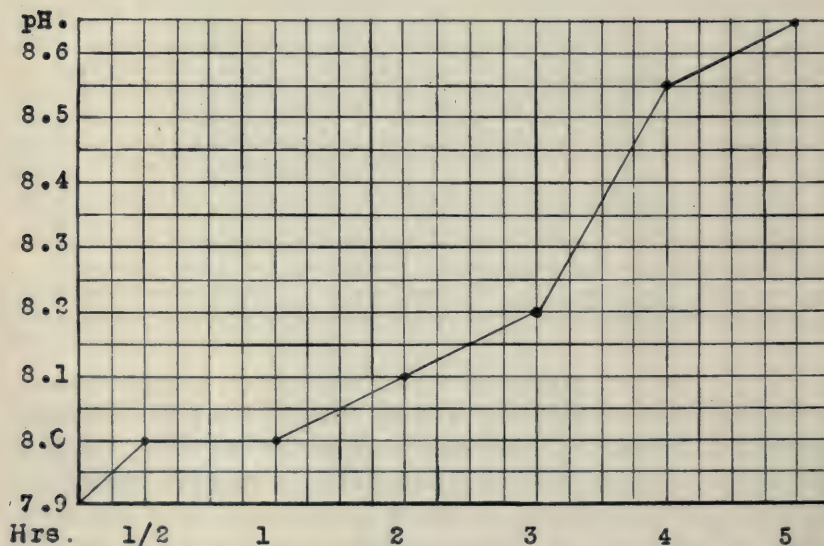


Chart 2.—Spinal Fluid 21-B. This curve shows the increase in alkalinity of the fluid after its withdrawal. The fluid was exposed to the atmosphere of the laboratory at irregular intervals for varying lengths of time. The change in the reaction occurred according to a correspondingly irregular curve.

TABLE 2.—NORMAL CEREBROSPINAL FLUIDS (ALL REACTIONS NORMAL)

Case No.	Diagnosis	Spinal Fluid					Blood Serum		
		Reac. (Immed.)		RpH	Cells	Globulin		Colloidal Gold	pH
		pH before Dial.	pH after Dial.			R-J	Pandy		
2-Z	Alcoholism.....	7.9	7.8	8.2	2	0	0	111000000	7.75
3-C	Dementia praecox.....	1.8	7.7	8.2	3	0	0	111111000	7.9
6-K	Manic depressive.....	7.8	7.8	8.2	3	0	0	000111000	7.75
7-O	Narcotism.....	8.1	7.75	8.2	4	0	0	1111110000	7.75
9-H	Constit. defective.....	7.8	7.75	8.2	5	0	0	11110000	7.7
10-R	Constit. defective.....	7.9	7.9	8.2	6	±	0	012222100	7.7
11-B	Manic depressive.....	7.8	7.8	8.4	...	+	±	001100000	7.7
14-E	Homosexual.....	7.8	7.7	8.6	2	0	0	001110000	7.75
15-S	Dementia praecox.....	7.85	7.8	8.4	5	0	0	1111110000	7.7
19-F	Dementia praecox.....	7.85	7.7	8.4	3	±	0	001111000	7.7
21-B	Alcoholism; Imbecility.....	7.9	7.7	...	5	0	0	00110000	...
23-E	Imbecility.....	7.85	7.8	...	3	0	0	000111000	...
25-D	Dementia praecox.....	7.7	7.7	...	5	0	0	110000000	...
27-G	Congenital defective.....	7.7	7.7	...	4	0	0	0011110000	...
33-H	Imbecility.....	7.75	7.65	...	5	0	0	000000000	7.7
36-C	Dementia praecox.....	7.75	7.8	...	4	0	0	011100000	7.65
37-G	Melancholia.....	7.75	7.7	...	10	0	±	000111000	7.8
39-M	Dementia praecox.....	7.9	7.9	...	5	0	0	000111000	7.7
40-W	Alcoholism.....	7.7	7.75	...	8	0	+	000111000	7.65
41-G	Dementia praecox.....	7.95	7.75	...	6	0	0	000111000	7.85
42-Z	Paranoia.....	7.9	7.7	...	4	0	±	000000000	7.6
44-S	Manic depression.....	7.7	7.75	...	3	+	0	000111000	7.7
45-S	Dementia praecox.....	7.75	7.7	...	5	0	0	000111000	7.8
47-B	Dementia praecox.....	7.8	7.75	...	4	+	0	000000000	7.8
48-S	Dementia praecox.....	7.7	7.75	...	3	0	0	000000000	7.7
50-G	Imbecility.....	7.7	7.7	...	2	0	0	011110000	7.7
54-M	Arteriosclerosis; hypertension.....	7.75	7.7	8.4	3?	+	+0	001110000	7.75



TABLE 3.—RESULTS OF CEREBROSPINAL FLUID TESTS IN SENILE-NORMAL CLASS

Case No.	Diagnosis	Spinal Fluid						Blood-Serum			
		Reac. (Immed.)		Cells	Globulin		Wasser- mann Reac- tion	Colloidal Gold	pH	Rph	Wasser- mann Reac- tion
		pH before Dial.	pH after Dial.		R-J	Pandy					
53-T	Hemiplegia (old); arteriosclerosis...	7.7	7.65	2	++	±	0	0 1 1 2 2 1 0 0 0	7.65	8.4	0
55-G	Amiotrophic lateral sclerosis.....	7.8	7.6	2	+	+	0	0 1 2 2 2 1 0 0 0	7.8	8.6	0
56-P	Hemiplegia (2 mo.); mitral insuf...	7.8	7.6	3	++	+++	0	0 0 1 1 1 1 0 0 0	...	...	0
57-T	Friedreich's ataxia.....	7.8	7.7	4	+	++	0	0 0 1 1 0 0 0 0 0	7.75	8.6	0
58-D	Paget's disease.....	7.75	7.7	2	+	±	0	0 0 1 1 1 1 0 0 0	7.75	8.4	0
59-B	Chr. bronchitis; arteriosclerosis...	7.8	7.75	3	+	++	0	0 1 2 2 2 1 0 0 0	8.0	8.2	0
60-C	Asthma, cardiorenal.....	7.7	7.7	3	+	++	0	0 0 1 1 1 0 0 0 0	7.85	8.4	0
61-D	Chronic alcoholism.....	7.7	7.65	5	+	±	0	1 1 1 1 1 0 0 0 0	7.85	8.6	0
62-F	Chr. arthritis; arteriosclerosis.....	7.8	7.6	2	++	++	0	1 1 1 1 1 0 0 0 0	7.9	8.6	0
63-S	Paramyoclonus.....	7.8	7.5	3	++	++	0	0 0 0 1 1 0 0 0 0	...	...	0
64-K	Cerebral arteriosclerosis.....	7.75	7.7	1	+	+	0	0 0 0 1 2 2 2 1 0	...	...	0
65-E	Arteriosclerosis.....	7.65	7.65	2	++	+	0	0 0 0 0 0 0 0 0 0	7.8	8.4	0
66-S	Chr. infect. arthritis.....	7.7	7.6	3	+	+	0	0 0 1 1 1 0 0 0 0	7.9	8.4	0
67-W	Hemiplegia (several years).....	7.85	7.7	2	+	±	0	0 0 1 1 1 1 0 0 0	7.85	8.2	0
68-B	Arteriosclerosis; nortic insuf.....	7.75	7.7	6	++	+++	0	0 0 1 2 2 2 0 0 0	7.85	8.4	0
69-H	Lateral sclerosis.....	7.7	7.6	5	++	++++	0	1 1 2 2 3 2 1 0 0	7.85	8.2	0
70-L	Chr. infect. arthritis.....	7.7	7.65	2	+	+	0	0 1 2 2 1 0 0 0 0	7.9	8.6	0
71-B	Paralysis agitans; Dercum's dis...	7.7	7.65	5	+	+	0	0 1 1 1 1 1 0 0 0	7.65	8.4	0
73-R	Chronic arthritis.....	7.75	7.7	5	±	+	0	1 1 1 1 1 0 0 0 0	...	...	0
75-S	Syringomyelia.....	7.75	7.7	6	+	+	0	1 1 1 1 1 0 0 0 0	7.7	8.4	0
76-W	Arterioscl.; hemiplegia (recent)....	7.75	7.7	3							

TABLE 4.—CEREBROSPINAL FLUID IN EPILEPSY

Case No.	Diagnosis	Spinal Fluid						Blood Serum				
		Reac. (Immed.)		RpH	Cells	Globulin		Wasser- mann Reac- tion	Colloidal Gold	pH	RpH	Wasser- mann Reac- tion
		pH before Dial.	pH after Dial.			R-J	Pandy					
8-H	Epilepsy.....	7.8	7.7	8.0	4	0	0	0	1111100000	7.6	8.1	0
24-B	Epilepsy.....	7.8	7.75	...	4	0	0	0	0001100000	...	...	0
26-G	Epilepsy.....	7.7	7.7	...	2	0	0	0	1110000000	...	...	0
43-B	Epilepsy.....	7.7	7.7	...	10	0	+	0?	0011220000	7.7	...	0
49-H	Epilepsy.....	7.8	7.75	...	4	±	±	0	1111000000	7.6	...	0
74-T	Epilepsy.....	7.65	7.6	8.6	4	+	+	0	1111100000	7.7	8.2	0
77-T	Epilepsy.....	7.7	7.65	8.4	+	+	0	0	1122110000	7.8	8.4	0

TABLE 5.—CEREBROSPINAL FLUID IN SYPHILIS

Case No.	Diagnosis	Spinal Fluid						Blood Serum	
		Reac. (Immed.)		R-PH	Cells	Globulin		Colloidal Gold	Wasser- mann Reaction, per Cent. +
		pH before Dial.	pH after Dial.			R-J	Pandy		
1-A	Paresis.....	7.8	7.7	8.2	13	+	±	3 3 2 2 1 1 0 0 0	50
13-B	Cerebrospinal syphilis.....	7.75	7.75	8.6	76	++	++	5 5 5 4 3 3 1 0 0	100
20-M	Paresis.....	7.95	7.9	8.4	14	+	+	5 5 5 3 1 1 1 0 0	100
28-P	Paresis.....	7.9	7.8	...	17	++	++	5 5 5 5 4 3 1 0 0	100
29-B	Paresis.....	7.7	7.7	...	21	++	++	5 5 5 5 5 4 3 2 1	100
30-V	Paresis.....	7.7	7.7	...	19	+++	+++	5 5 5 5 5 5 5 4 3	100
32-C	Paresis.....	7.75	7.7	...	16	++	++	3 3 3 3 + 4 4 3 2 1	100
72-O	Arteriosclerosis; hemiplegia (old)...	7.75	7.7	8.6	12	+++	++	4 4 4 4 4 3 2 1 0	100
4-S	Dementia praecox; syphilis.....	8.2	7.7	8.0	0	0	0	1 1 2 2 2 1 0 0 0	0
31-P	Cerebrospinal syphilis.....	7.8	7.7	...	4	0	0	0 0 1 1 1 0 0 0 0	100
34-T	Paresis.....	7.8	7.7	...	19	+	++	3 3 3 3 + 4 4 3 2 1	100
38-W	Cerebrospinal syphilis.....	7.8	7.8	...	48	++	++	0 0 0 2 3 3 3 2 1	100
51-B	Cerebrospinal syphilis.....	7.8	7.75	8.4	18	++	++	0 1 2 3 3 2 1 0 0	100
5-K	Alcoholism.....	7.9	7.4	5.2	6	0	0	1 1 2 2 2 1 1 0 0	75
12-K	Syphilis; imbecile.....	7.8	7.7	8.6	4	±	0	0 1 1 1 1 0 0 0 0	75
16-P	Imbecile.....	7.85	7.8	8.4	Bloody fluid 3	+	±	Bloody fluid	25
17-H	Chronic alcoholism.....	7.8	7.8	8.4	11	±	±	0 0 0 0 0 0 0 0 0	75
18-L	Alcoholism; syphilis.....	7.8	7.75	8.4	4	±	0	0 1 1 1 1 0 0 0 0	100
22-B	Alcoholism.....	7.8	7.8	...	2	±	+	1 1 1 1 0 0 0 0	100
35-M	Alcoholism.....	7.7	7.75	...	5	0	0	0 0 1 1 1 0 0 0 0	100
52-C	Aortic insufficiency.....	7.7	...	8.4	5	+	±	0 1 1 2 2 2 1 1 1	100



## RESULTS

Table 2 summarizes the tests on cerebrospinal fluids considered to be normal—having no abnormal reactions. The low hydrogen ion concentration found in Case 7-C was probably due to an error in technic, as the reaction of the dialysate of this fluid was  $\text{pH} = 7.75$ . The pH of the other fluids included in this table averaged 7.8; the RpH from 0.4 to 0.6; while the pH the RpH of the blood serum in these cases averaged, respectively, 7.75 and 0.5 to 0.8.

Table 3 gives the results of the tests in the cases classifiable as "senile-normal," the fluids having been obtained from patients over 60 years of age. The interesting abnormality in these fluids was the presence of an amount of albumin and globulin precipitable by the Ross-Jones and Pandy tests without coincident increase of cells or decolorization of the colloidal gold. The fluids were otherwise normal, and showed approximately the same pH and RpH as the cases comprising Table 2.

Table 4 contains the group of epileptics. The hydrogen ion concentration of these fluids and the blood serum from these patients were 7.65 to 7.8, as in the normal cases.

Table 5 contains the results of the tests in twenty-one cases of several types of syphilis of the central nervous system, having a positive Wassermann reaction in the blood, spinal fluid, or in both. In the presence of the increased numbers of cells and abnormal amounts of protein in these fluids, the pH varied between 7.7 and 8.2; and 7.4 and 7.9 in their dialysates. This type of pathologic process apparently has no influence on the reaction of the cerebrospinal fluid, and hydrogen ion concentration here is within normal limits.

## CONCLUSIONS

These studies allow us to draw the following conclusions:

1. To obtain the true hydrogen ion concentration of the cerebrospinal fluid it is necessary to test the fluid immediately on its withdrawal from the body, avoiding as much as possible exposure to the air.
2. The colorimetric method for determining the hydrogen ion concentration is found to have certain limitations, due chiefly to the peculiar color shades produced by the carbon dioxid content of the spinal fluid. Aside from the differences which may exist between the values of the pH determined by this method and the gas-chain method, the results obtained by the colorimeter have slight inaccuracies, probably equivalent to 0.1 when the hydrogen ion concentration lies between 7.5 and 7.7.

3. When the hydrogen ion concentration of the spinal fluid is determined by the colorimetric method, using phenolsulphonephthalein as the indicator, the values obtained are: average pH of the cerebrospinal fluid = 7.75, with maximum variations of 7.4 to 7.9. The RpH varies from 0.4 to 0.6.

4. This confirms the work of Bisgaard, in which he states that the reaction of the cerebrospinal fluid is more acid than  $\text{pH} = 8.1$ .

5. The hydrogen ion concentration of the blood serum and of the spinal fluid are approximately equal.

1097

## INDEX TO VOLUME XIX

	PAGE
Addis, T.: Causes of the variation in the concentration of urea in the blood of young, healthy adults.....	507
Albuminuria and renal functional changes following administration of full therapeutic doses of salicylate; P. J. Hanzlik, R. W. Scott and T. W. Thoburn .....	1029
Alcohol, human and animal liver after; F. A. McJunkin.....	786
Alcohol, infundibular tumor in a child causing diabetes insipidus with tolerance of; L. Newmark.....	550
Ambard's coefficient of urea excretion, clinical value of; D. Sclater Lewis..	1
Amino-acids, present significance of, in physiology and pathology; Donald D. Van Slyke.....	56
Anemia, aplastic, purpura hemorrhagica, and allied conditions, classification and diagnosis of. Diminished blood platelets and marrow insufficiency; G. R. Minot.....	1062
Anemia, influence of splectomy on metabolism in; W. Denis.....	344
Anemia, pernicious, bile content of blood in; M. A. Blankenhorn.....	709
Appendicitis, experimental; J. W. McMeans.....	153
Arthritis, experimental, effect of heat and continuous incandescent electric light in; W. E. Simmonds.....	529
Arthritis, experimental, in rabbits, effect of salicylates on; B. Fantus, W. E. Simmonds and J. J. Moore.....	823
Aub, J. C.: Clinical calorimetry. Nineteenth paper. The basal metabolism of old men.....	832
Aub, J. C.: Clinical Calorimetry. Twentieth paper. The effect of caffen on heat production.....	840
Aub, J. C.: Clinical calorimetry. Twenty-first paper. Basal metabolism of dwarfs and legless men, with observations on the specific dynamic action of proteins.....	865
Aub, J. C.: Clinical calorimetry. Twenty-second paper. The respiratory metabolism in nephritis.....	890
Aub, J. C.: Clinical calorimetry. Twenty-third paper. The effect of Roentgen-ray and radium therapy on the metabolism of a patient with lymphatic leukemia.....	908
Aub, J. C.: Clinical calorimetry. Twenty-fourth paper. Metabolism in three unusual cases of diabetes.....	695
Barnett, G. D.: Intestinal eosinophilia, with report of a case.....	1085
Bayne-Jones, S.: Reaction of the cerebrospinal fluid. Preliminary report on the hydrogen-ion concentration as determined by the colorimetric method .....	538
Bayne-Jones, S.: Roentgenography of the Lungs. Roentgenographic studies in living animals after intratracheal injections of iodoform emulsion..	397
Berkowitz, S.: Malone-Kiutsi reactions in pregnancy and cancer.....	344
Blankenhorn, M. A.: Bile content of blood in pernicious anemia.....	767
Blood and alveolar air, carbon dioxid content of, in obstructed expiration; E. D. Friedman and H. C. Jackson.....	344
Blood, bile content of, in pernicious anemia; M. A. Blankenhorn.....	105
Blood changes in albino rats following removal of spleen; C. C. Wolferth..	335
Blood flow in feet, effect of bandaging of legs on rate of; G. N. Stewart...	507
Blood of young, healthy adults, causes of the variation in the concentration of urea in; T. Addis and C. K. Watanabe.....	



# INDEX TO VOLUME XIX

	PAGE
Blood Platelets, diminished, and marrow insufficiency. A classification and differential diagnosis of purpura hemorrhagica, aplastic anemia and allied conditions; G. R. Minot.....	1062
Blood pressure, systolic, following exercise, with remarks on cardiac capacity; D. L. Rapport.....	981
Blood sugar, a study of. A comparison of the tolerance for glucose in diabetic and normal subjects; R. Cummings and G. Piness.....	777
Caffein, effect of, on heat production. Clinical calorimetry. Twentieth paper. J. H. Means, J. C. Aub and E. F. DuBois.....	832
Calorimetry, clinical. Nineteenth paper. The basal metabolism of old men; J. C. Aub and E. F. DuBois.....	823
Calorimetry, clinical. Twentieth paper. The effect of caffein on heat production; J. H. Means, J. C. Aub, and E. F. DuBois.....	832
Calorimetry, clinical. Twenty-first paper. The basal metabolism of dwarfs and legless men, with observations on the specific dynamic action of proteins; J. C. Aub and E. F. DuBois.....	840
Calorimetry, clinical. Twenty-second paper. The respiratory metabolism in nephritis; J. C. Aub and E. F. DuBois.....	865
Calorimetry, clinical. Twenty-third paper. The effect of Roentgen-ray and radium therapy on the metabolism of a patient with lymphatic leukemia; J. B. Murphy, J. H. Means and J. C. Aub.....	890
Calorimetry, clinical. Twenty-fourth paper. Metabolism in three unusual cases of diabetes; F. C. Gephart, J. C. Aub, E. F. DuBois and Graham Lusk.....	908
Calorimetry, clinical. Twenty-fifth paper. Water elimination through the skin and respiratory passages in health and disease; G. F. Soderstrom and E. F. DuBois.....	931
Carbohydrate metabolism, the suprarenal system and; G. M. Mackenzie....	593
Carbon dioxid content of blood and of alveolar air in obstructed expiration; E. D. Friedman and H. C. Jackson.....	767
Cardiac capacity; systolic blood pressure following exercise, with remarks on; D. L. Rapport.....	981
Cardiac impulse, experiments on origin and conduction of. VI. Sinoven-tricular and sino-auricular heart-block; J. A. E. Eyster and W. J. Meek	117
Cerebral cortex, influence of radiations from Kromayer's mercury quartz lamp on; H. Wago.....	801
Cerebrospinal fluid, reaction of. Preliminary report on hydrogen-ion concentration as determined by the colorimetric method; L. D. Felton, R. G. Hussey and S. Bayne-Jones.....	1085
Chesney, A. M.: A study of ethylhydrocuprein (optochin) in the treatment of acute lobar pneumonia.....	611
Circulation, the reflex action of volatile irritants on; C. C. Lieb and W. W. Herrick.....	811
Cummings, R.: A study of blood sugar. A comparison of the tolerance for glucose in diabetic and normal subjects.....	777
Diabetes insipidus with tolerance of alcohol, a case of infundibular tumor in a child causing; L. Newmark.....	550
Diabetes, metabolism in three unusual cases of. Clinical calorimetry, Twenty-fourth paper; F. C. Gephart, J. C. Aub, E. F. DuBois and Graham Lusk.....	908
DuBois, E. F.: Clinical calorimetry. Nineteenth paper. The basal metabolism of old men.....	823
DuBois, E. F.: Clinical calorimetry. Twentieth paper. The effect of caffein on heat production.....	832
DuBois, E. F.: Clinical calorimetry. Twenty-first paper. Basal metabolism of dwarfs and legless man.....	840

# INDEX TO VOLUME XIX

1099

	PAGE
DuBois, E. F.: Clinical calorimetry. Twenty-second paper. The respiratory metabolism in nephritis.....	865
DuBois, E. F.: Clinical calorimetry. Twenty-fourth paper. Metabolism in three unusual cases of diabetes.....	908
DuBois, E. F.: Clinical calorimetry. Twenty-fifth paper. Water elimination through the skin and respiratory passages in health and disease...	931
Dick, George F.: Bacteriology of urine in focal infections; its relation to nephritis .....	493
Dick, Gladys R.: Bacteriology of urine in focal infections; its relation to nephritis .....	493
Eosinophilia, intestinal, with report of a case; G. D. Barnett.....	695
Endocarditis, subacute streptococcus, bacteriologic studies in; R. A. Kinsella	367
Ethylhydrocuprein (optochin) in the treatment of acute lobar pneumonia; H. F. Moore and A. M. Chesney.....	611
Expiration, obstructed, carbon dioxid content of blood and of alveolar air in; E. D. Friedman and H. C. Jackson.....	767
Eyster, J. A. E.: Experiments on origin and conduction of cardiac impulse. VII. Sinoventricular and sino-audicular heart-block.....	117
Fantus, B.: Effect of salicylates on experimental arthritis in rabbits.....	529
Fat cells, embryonal, the significance of, in certain pathologic conditions. D. Symmers and A. Fraser.....	699
Felton, L. D.: Reaction of the cerebrospinal fluid. Preliminary report on hydrogen-ion concentration as determined by the colorimetric method..	1085
Female remedies, action of several, on strips of excised human uterus; J. D. Pilcher.....	53
Force, J. N.: Further studies on typhoidin.....	440
Fraser, A.: The significance of embryonal fat cells in certain pathologic conditions .....	699
Friedman, E. D.: The carbon dioxid content of blood and of alveolar air in obstructed expiration.....	767
Garrison, P. E.: An experimental test of the relation of sewage disposal to the spread of pellagra.....	683
Garrison, P. E.: Relation of pregnancy and childbirth to pellagra in women	404
Gephart, F. C.: Clinical calorimetry. Twenty-fourth paper. Metabolism in three unusual cases of diabetes.....	890
Gilbert, Quinter O.: Occurrence of nuclear changes in red blood cells following splenectomy.....	140
d-Glucose tolerance in health and disease; Russell M. Wilder and W. D. Sansum .....	311
Haller, D. A.: Treatment of syphilis of the central nervous system. A comparison of mercurialized serum and salvarsanized serum.....	997
Hanzlik, P. J.: The salicylates. VI. Renal function and morphologic changes in animals following the administration of.....	1016
Hanzlik, P. J.: The salicylates. VII. Further observations on albuminuria and renal functional changes following the administration of full therapeutic doses of.....	1029
Hay-fever and hay-fever pollens; William Scheppegegrell.....	959
Heart-block, sinoventricular and sino-audicular. VII. Experiments on origin and conduction of cardiac impulse; J. A. E. Eyster and W. J. Meek...	117
Heart Block, transient—electrocardiographic studies; E. B. Krumbhaar...	750
Herrick, W. W.: The reflex action of volatile irritants on the circulation..	811
Hess, J. H.: Autotransplantation and homotransplantation of the thyroid gland, using the capsule as the seat of transplantation.....	518
Hess, J. H.: Osteogenesis imperfecta.....	163
Hodgkin's disease, a new interpretation of the pathologic histology of; D. Symmers.....	990



# INDEX TO VOLUME XIX

	PAGE
Hoxie, G. H.: The reactivated thymus.....	564
Hussey, R. G.: Reaction of the cerebrospinal fluid. Preliminary report on the hydrogen-ion concentration as determined by the colorimetric method .....	1085
Hydrogen-ion concentration in the cerebrospinal fluid as determined by the colorimetric method, preliminary report on; L. D. Felton, R. G. Hussey and S. Bayne-Jones.....	1085
Infections, focal, bacteriology of urine in; its relation to nephritis; George F. Dick and Gladys R. Dick.....	493
Infections, influence of nonspecific substances on; J. W. Jobling.....	1042
Iodoform emulsion, roentgenographic studies in living animals after intratracheal injections of. Roentgenography of the lungs; C. A. Waters, S. Bayne-Jones and L. G. Rowntree.....	538
Irritants, volatile, reflex action of, on the circulation; C. C. Lieb and W. W. Herrick .....	811
Jackson, H. C.: The carbon dioxid content of blood and of alveolar air in obstructed expiration.....	767
Jobling, J. W.: Influence of nonspecific substances on infections.....	1042
Karsner, H. T.: The salicylates. VI. Renal function and morphologic changes in animals following the administration of.....	1016
Kessel, Leo: Relation of hypertrophic osteo-arthritis to pulmonary tuberculosis .....	239
Kilgore, Eugene S.: A comparison of two methods of vaccination against typhoid fever.....	276
Kilgore, Eugene S.: Agglutinins and complement-fixing antibodies in serum of persons vaccinated against typhoid fever.....	293
Kilgore, Eugene S.: Typhoidin quotients. An analysis of factors of uncertainty in cutaneous typhoidin test.....	263
Kinsella, Ralph A.: Bacteriologic studies in acute rheumatic fever.....	381
Kinsella, Ralph A.: Bacteriologic studies in subacute streptococcus endocarditis .....	367
Kromayer's mercury quartz lamp, influence of radiations from, on the cerebral cortex (animal experiments); H. Wago.....	801
Krumbhaar, E. B.: Transient heart block, electrocardiographic studies....	750
Leukemia, lymphatic, the effect of Roentgen-ray and radium therapy on the metabolism of a patient with. Clinical calorimetry. Twenty-third paper; J. B. Murphy, J. H. Means and J. C. Aub.....	890
Lewis, D. Sclater: Clinical value of Ambard's coefficient of urea excretion .....	1
Lieb, C. C.: The reflex action of volatile irritants on the circulation.....	811
Liver, the human and animal, after alcohol; F. A. McJunkin.....	786
Lungs, roentgenography of. Roentgenographic studies in living animals after intratracheal injections of iodoform emulsion; C. A. Waters, S. Bayne-Jones and L. G. Rowntree.....	538
Lusk, Graham: Clinical calorimetry. Twenty-fourth paper. Metabolism in three unusual cases of diabetes.....	908
Mackenzie, G. M.: The suprarenal system and carbohydrate metabolism... ..	593
MacNeal, W. J.: An experimental test of the relation of sewage disposal to the spread of pellagra.....	683
MacNeal, W. J.: Relation of pregnancy and childbirth to pellagra in women .....	404
Malone-Kiutsi reactions in pregnancy and cancer; Samuel Berkowitz.....	397
McClure, C. W.: A study of the diastatic activity of the urine and feces, with special reference to diseases of the pancreas.....	568
McMeans, J. W.: Experimental appendicitis.....	709
Means, J. H.: Clinical calorimetry. Twentieth paper. The effect of caffeine on heat production.....	832



# INDEX TO VOLUME XIX

1101

	PAGE
Means, J. H.: Clinical calorimetry. Twenty-third paper. The effect of Roentgen-ray and radium therapy on the metabolism of a patient with lymphatic leukemia.....	890
Meek, W. J.: Experiments on origin and conduction of cardiac impulse. VII. Sinoventricular and sino-auricular heart-block.....	117
Metabolism, basal, of dwarfs and legless men, with observations on the specific dynamic action of proteins. Clinical calorimetry. Twenty-first paper; J. C. Aub and E. F. DuBois.....	840
Metabolism, basal, of old men. Clinical calorimetry. Nineteenth paper; J. C. Aub and E. F. DuBois.....	823
Metabolism in three unusual cases of diabetes. Clinical calorimetry. Twenty-fourth paper; F. C. Gephart, J. C. Aub, E. F. DuBois and Graham Lusk.....	908
Metabolism of a patient with lymphatic leukemia, the effect of Roentgen-ray and radium therapy on. Clinical calorimetry. Twenty-third paper; J. B. Murphy, J. H. Means and J. C. Aub.....	890
Metabolism, respiratory, in nephritis. Clinical calorimetry. Twenty-second paper; J. C. Aub and E. F. DuBois.....	865
Meyer, K. F.: Agglutinins and complement-fixing antibodies in serum of persons vaccinated against typhoid fever.....	293
Minot, G. R.: Diminished blood platelets and marrow insufficiency. A classification and differential diagnosis of purpura hemorrhagica, aplastic anemia and allied conditions.....	1062
Moore, H. F.: A study of ethylhydrocuprein (optochin) in the treatment of acute lobar pneumonia.....	611
Moore, J. J.: Effect of heat and continuous incandescent electric light in experimental arthritis.....	153
Moore, J. J.: Effect of salicylates on experimental arthritis in rabbits.....	529
Murphy, J. B.: Clinical calorimetry. Twenty-third paper. The effect of Roentgen-ray and radium therapy on the metabolism of a patient with lymphatic leukemia.....	890
Muscular dystrophy, progressive, as an endocrine disease; W. Timme.....	79
Muscular work, diet and hemolysis, the effect of on the serum proteins; A. H. Rowe.....	499
Nephritis, the respiratory metabolism in. Clinical calorimetry. Twenty-second paper; J. C. Aub and E. F. DuBois.....	865
Nephritis, refractometric studies of serum proteins in nephritis, cardiac decompensation, diabetes, anemia and other chronic disease; A. H. Rowe .....	354
Newmark, L.: A case of infundibular tumor in a child causing diabetes insipidus with tolerance of alcohol.....	550
Osteo-arthropathy, hypertrophic, relation of, to pulmonary tuberculosis; L. Kessel .....	239
Osteogenesis imperfecta; J. H. Hess.....	163
Ottenberg, R.: On reliability of the Wassermann reaction. Study of sources of error and attempt to standardize technic.....	457
Pancreas, a study of the diastatic activity of the urine and feces, with special reference to diseases of; C. W. McClure and J. H. Pratt.....	568
Pellagra, an experimental test of the relation of sewage disposal to the spread of; J. F. Siler, P. E. Garrison and W. J. MacNeal.....	683
Pellagra in women, relation of pregnancy and childbirth to; J. F. Siler, P. E. Garrison and W. J. MacNeal.....	404
Pigments, blood-derived, duodenal, further quantitative study of; J. P. Schneider .....	156
Pilcher, J. D.: Action of several "female remedies" on strips of excised human uterus .....	53

# INDEX TO VOLUME XIX

	PAGE
Piness, G.: A study of blood sugar. A comparison of the tolerance for glucose in diabetic and normal subjects.....	777
Pituitary injections, effect of, on blood pressure of febrile patients; H. B. Schmidt .....	1059
Pneumonia, acute lobar, a study of ethylhydrocuprein (optochin) in the treatment of; H. F. Moore and A. M. Chesney.....	611
Pratt, J. H.: A study of the diastatic activity of the urine and feces, with special reference to diseases of the pancreas.....	568
Pregnancy and cancer, Malone-Kiutsi reactions in; Samuel Berkowitz.....	397
Proteins, basal metabolism of dwarfs and legless men, with observations on the specific dynamic action of. Clinical calorimetry. Twenty-first paper; J. C. Aub and E. F. DuBois.....	840
Purpura hemorrhagica, aplastic anemia and allied conditions, a classification and differential diagnosis of. Diminished blood platelets and marrow insufficiency; G. R. Minot.....	1062
Pylorus, clinical study of secretions on proximal and distal sides of; A. S. Robinson .....	220
Rapport, D. L.: The systolic blood pressure following exercise; with remarks on cardiac capacity.....	981
Renal function and morphologic changes in animals following the administration of salicylate; P. J. Hanzlik and H. T. Karsner.....	1016
Rheumatic fever, acute, bacteriologic studies in; H. F. Swift and R. A. Kinsella .....	381
Robinson, A. S.: Clinical study of secretions on proximal and distal sides of pylorus .....	220
Rowe, A. H.: Refractometric studies of serum proteins in nephritis, cardiac decompensation, diabetes, anemia, and other chronic diseases.....	354
Rowe, A. H.: The effect of muscular work, diet and hemolysis on the serum proteins.....	499
Rowntree, L. G.: Roentgenography of the lungs. Roentgenographic studies in living animals after intratracheal injections of iodoform emulsion..	538
Salicylates. VI: Renal function and morphologic changes in animals following the administration of; P. J. Hanzlik and H. T. Karsner...	1016
Salicylates. VII. Further observations on albuminuria and renal functional changes following the administration of full therapeutic doses of; P. J. Hanzlik, R. W. Scott and T. W. Thoburn.....	1029
Salicylates, the effect of, on experimental arthritis in rabbits; B. Fantus, W. E. Simmonds and J. J. Moore.....	529
Sansum, W. D.: d-Glucose tolerance in health and disease.....	311
Scheppegrell, W.: Hay-fever and hay-fever pollens.....	959
Schmidt, H. B.: Effect of pituitary injections on the blood pressure of febrile patients.....	1059
Schneider, J. P.: Further quantitative study of duodenal blood-derived pigments .....	156
Scott, R. W.: The salicylates. VII. Further observations on albuminuria and renal functional changes following the administration of full therapeutic doses of.....	1029
Serum proteins, refractometric studies of, in nephritis, cardiac decompensation, diabetes, anemia and other chronic diseases; A. H. Rowe.....	354
Serum proteins, the effect of muscular work, diet and hemolysis on; A. H. Rowe.....	499
Sewage disposal, an experimental test of the relation of, to the spread of pellagra; J. F. Siler, P. E. Garrison and W. J. MacNeal.....	683
Siler, J. F.: An experimental test of the relation of sewage disposal to the spread of pellagra.....	683
Siler, J. F.: Relation of pregnancy and childbirth to pellagra in women...	404



# INDEX TO VOLUME XIX

1103

	PAGE
Simmonds, W. E.: Effect of salicylates on experimental arthritis in rabbits	529
Simmonds, W. E.: Effect of heat and continuous incandescent electric light in experimental arthritis.....	153
Soderström, G. F.: Clinical Calorimetry. Twenty-fifth paper. Water elimination through skin and respiratory passages in health and disease	931
Spleen, blood changes in albino rats following removal of; C. C. Wolferth	105
Splenectomy, occurrence of nuclear changes in red blood cells following; Q. O. Gilbert.....	140
Steiner, Walter R.: Hereditary hemorrhagic telangiectasia, with report of three families and review of those previously recorded.....	194
Stevens, Ida M.: Further studies on typhoidin.....	440
Stewart, G. N.: Effect of bandaging of legs on rate of blood flow in feet..	335
Strauss, A. A.: Autotransplantation and homotransplantation of the thyroid gland, using the capsule as the seat of transplantation.....	518
Suprarenal system and carbohydrate metabolism; G. M. Mackenzie.....	593
Swift, Homer F.: Bacteriologic studies in acute rheumatic fever.....	381
Symmers, D.: A new interpretation of the pathologic histology of Hodgkin's disease.....	990
Symmers, D.: The significance of embryonal fat cells in certain pathologic conditions .....	699
Syphilis, treatment of, of the central nervous system. A comparison of mercurialized serum and salvarsanized serum; D. A. Haller.....	997
Telangiectasia, hereditary hemorrhagic, with report of three families and review of those previously recorded; W. R. Steiner.....	194
Thoburn, T. W.: The salicylates. VII. Further observations on albuminuria and renal functional changes following the administration of full therapeutic doses of.....	1029
Thymus, the reactivated; G. H. Hoxie.....	564
Thyroid gland, autotransplantation and homotransplantation of, using the capsule as the seat of transplantation; J. H. Hess and A. A. Strauss...	518
Timme, W.: Progressive muscular dystrophy as an endocrine disease.....	79
Tuberculosis, pulmonary, relation of hypertrophic osteo-arthritis to; L. Kessel .....	239
Tumor, infundibular, in a child, causing diabetes insipidus with tolerance of alcohol; L. Newmark.....	550
Typhoid fever, agglutinins and complement fixing antibodies in serum of persons vaccinated against; K. F. Meyer and Eugene S. Kilgore.....	293
Typhoid fever, comparison of two methods of vaccinating against; E. S. Kilgore .....	276
Typhoidin, further studies on; J. N. Force and Ida M. Stevens.....	440
Typhoidin quotients. An analysis of factors of uncertainty in cutaneous typhoidin test; E. S. Kilgore.....	263
Urea excretion, clinical value of Ambard's coefficient of; D. Slater Lewis	1
Urea in the blood of young, healthy adults, causes and variation in the concentration of; T. Addis and C. K. Watanabe.....	507
Urine and feces, diastatic activity of, with special reference to disease of the pancreas; C. W. McClure and J. H. Pratt.....	568
Urine, bacteriology of, in focal infections; its relation to nephritis; George F. Dick and Gladys R. Dick.....	493
Van Slyke, Donald D.: Present significance of amino-acids in physiology and pathology.....	56
Wago, H.: Influence of the radiations from Kromayer's mercury quartz lamp on the cerebral cortex.....	801
Wassermann reaction, on the reliability of. Study of the sources of error and attempt to standardize technic; R. Ottenberg.....	457



# INDEX TO VOLUME XIX

	PAGE
Wilder, Russell M.: d-Glucose tolerance in health and disease.....	311
Watanabe, C. K.: Causes of the variation in the concentration of urea in the blood of young, healthy adults.....	507
Waters, C. A.: Roentgenography of the lungs. Roentgenographic studies in living animals after intratracheal injections of iodoform emulsion..	538
Water elimination through skin and respiratory passages in health and disease. Clinical calorimetry. Twenty-fifth paper; G. F. Soderstrom and E. F. DuBois.....	931
Wolferth, C. C.: Blood changes in albino rats following removal of spleen	105









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